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# EFFECT OF SPRINKLER IRRIGATION IN AN ARID CLIMATE ON THE SPREAD OF BACTERIAL DISEASES OF BEANS[1]

J. D. Menzies[2]

SUMMARY

The effect of overhead sprinkler irrigation on the amount of bacterial disease damage to beans was studied under the typically arid climate of the Columbia Basin region of Washington.

None of the 4 bacterial diseases that were observed occurs in this area on beans irrigated by the furrow method but all have appeared late in the growing season on sprinkled fields. Experiments were conducted principally with *Xanthomonas phaseoli*, the cause of common bacterial blight.

Using artificially established infection centers in fields irrigated by sprinkler for various time intervals, it was found that practically no spread of blight infection was obtained during July and August with up to 30 hours of continuous irrigation. In September, as the crop approached maturity, disease sometimes developed rapidly but because of late occurrence the damage was negligible.

It is concluded that in the normal climate of this area beans can be irrigated by overhead sprinkling without serious danger from bacterial diseases provided they are planted early enough to mature before October.

In the irrigated regions of western United States sprinkler systems are being widely used particularly on sandy soils or where the topography is not suited to the furrow or rill method of irrigation. Since natural rainfall is generally conducive to fungus and bacterial foliage diseases, the question arises: to what extent will sprinkler irrigation favor these diseases? This report presents data on the spread of bacterial diseases of beans and lima beans as influenced by sprinkler irrigation in the Columbia Basin area of Central Washington. Artificial irrigation is, in this arid climate, a substitute for, rather than a supplement to, natural rain.

In comparing sprinkler and natural rain there are several differences, important to the spread of plant diseases, that should be noted. Natural rainstorms usually cover large land areas; they are usually preceded by a period of cloudy weather during which temperature falls and humidity increases. The rain is often accompanied by wind. After the rainfall there is a variable time of high humidity and cloudiness, during which temperatures again rise to the normal arid level.

Artificial sprinkler rain is usually applied suddenly under hot, arid conditions and as abruptly terminated. The small land area involved is surrounded by hot, dry air which immediately moves in over the sprinkled field if there is any wind movement. Free water on crop foliage may be gone within 30 minutes after sprinkling ceases during hot weather, and the temperature drop is of short duration.

Sprinklers are probably more effective in dissemination of inoculum than is gentle natural rain. This is especially true of the high pressure, low angle rotary sprinklers. They compare more to the heavy, beating rainstorms that are accompanied by wind. Large droplets of water are delivered at a driving angle to the plants and thus splash from plant to plant.

Bean diseases on which experimental data have been obtained are common bacterial blight caused by *Xanthomonas phaseoli* (E. F. Sm.) Dowson and bacterial leafspot of lima bean caused by *Pseudomonas syringae* v. Hall. Observations have also been made on the effect of sprinkling on halo blight, *Pseudomonas phaseolicola* (Burk.) Dows. and bacterial wilt *Corynebacterium flaccumfaciens* (Hedges) Dows. All were recorded in the Columbia Basin for the first time in 1947 except wilt, which was first found in the area in 1951. There was no commercial bean acreage in the area prior to 1946.

SPRINKLER VS. FURROW IRRIGATION.—*Materials and Methods.*—During the summer of 1950 a comparison was made between sprinkler and furrow irrigation as they influenced the spread of the bacteria causing common blight of bean and bacterial leafspot of lima bean from artificial inoculation centers. Both organisms were tested on Henderson Bush lima beans and common blight was used on Red Mexican field beans. The work was conducted on the Development Farm near Winchester, Washington, operated by the Bureau of Reclamation, U. S. Department of Interior.

The experimental area was divided into 2 blocks for the irrigation treatments. These were not replicated because of the cost and labor involved in installing pipe lines and waste-water ditches. The block to be furrow irrigated was placed 100 ft. to windward of the sprinkler block to protect it from sprinkler drift. Within each block the 3 crop-disease combinations were arranged in triplicate in a randomized block design, each plot being 60 ft. × 30 ft. in size. Beans were planted in 24-in. rows, allowing 15 rows per plot.

Sprinkler equipment consisted of 20° rotary sprinklers with 5/32 in. nozzles placed at 40-ft. intervals on 24-in. risers from 2-in. portable irrigation pipe. Plots

[1] Accepted for publication May 24, 1954.

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Acknowledgment is made to the Bureau of Reclamation personnel for their cooperation, to John S. Unbewust who was responsible for the irrigation applications, to C. E. Nelson for assistance and advice to the field operations, and to W. J. Zaumeyer and W. H. Burkholder for cultures of bacterial pathogens.

were sprinkled for 24 hours during an irrigation. To equalize as much as possible the water applications of the 2 systems of irrigation, corresponding strips in the furrow-irrigated plots were watered during each sprinkler setting.

All plots were irrigated by sprinklers prior to July 8 when differential treatments were begun. At this time the plants were about 6 in. high. Nine comparative irrigations were subsequently applied on the following schedule: July 8, 13, 18, 24 and 31, August 10, 21 and September 5 and 19. Natural rainfall during the entire test period amounted to only a trace recorded in August 14 and 15.

In order to assure a uniform source of primary infection, artificial infection centers were established at 4 locations in the middle row of each plot. Each center consisted of a 2-ft. strip of plants artificially inoculated with the selected organism on July 18. In making inoculations a water suspension from beef broth cultures of the test organism was sprayed on the plants by means of a small hand sprayer. Carborundum powder was used in the suspension to improve inoculation. The inoculated plants were covered with burlap during the July 18 irrigation to prevent washing off of the inoculum. Two weeks later primary infections were general at all inoculation points in both series.

*Results.*—During the entire observation period extending until September 19 no secondary infections from either pathogen could be found in the furrow irrigated plots. The primary infections caused by *P. syringae* on Henderson Bush lima beans consisted of small necrotic spots. No defoliation occurred and the original infections were difficult to locate by the end of the season. The common blight organism caused more extensive damage on both field beans and lima beans. Even without secondary spread the primary infections caused enough damage to defoliate seriously the plants in the infection centers.

Secondary infections occurred under sprinkler irrigation with all crop-disease combinations. Details of the disease spread are given below.

*Bacterial leaf spot on lima beans.*—Secondary lesions were first noted in lima beans 1 month after the original inoculation. These were confined to the inoculation centers, there being no spread beyond them even though 3 sprinkler irrigations of 24 hours each had been applied. When final observations were made on September 19 the following amount of spread was noted:

Replicate 1. Many new lesions on infected plants; spread occurred along inoculated row for 2 or 3 ft. in direction away from nearest sprinkler but only 6 in. towards sprinkler. A few lesions noted on adjacent rows but only a trace in second row over.

Replicate 2. No secondary lesions from either infection center and no remaining evidence of primary infection could be found at end of season.

Replicate 3. Down-row spread ranged from 1.5 to 3 ft., lateral spread to second row from inoculated row in direction away from nearest sprinkler but no lateral spread towards sprinkler.

*Common blight on lima beans.*—Secondary infections on lima beans by the common blight organism were more numerous and destructive under sprinklers than was the case with bacterial leaf spot.

Replicate 1. Down-row spread from inoculation centers ranged from less than 1 ft. to 21 ft., the greatest distance of spread being away from the nearest sprinkler.

Replicate 2. Down-row spread of 8 to 24 ft. from inoculation centers.

Replicate 3. Infections in inoculated row involved the entire 60 ft. of that row.

In all replicates, lateral spread was recorded to the second row from the nearest sprinkler but only to the adjacent row in the other direction. The inoculum was not successfully disseminated except where there was close plant contact. The driving action of the sprinklers caused some splashing of bacteria to adjacent rows and favored spread down the row away from the source of water.

*Common blight on field beans.*—Common blight spread extensively in the sprinkled plots of Red Mexican field beans. Spread from the original infection centers resulted in scattered secondary centers. Early in the season the blight progressed down the rows but by the time plant growth closed the space between rows lateral spread was rapid. By the end of the growing period the area of blight-infected plants was estimated visually as 10, 30, and 75 per cent respectively of the total area of the 3 replicates.

*Effect of length of sprinkling time on spread of bacterial blight.*—In the above experiment these bacterial diseases did not spread rapidly with a 24-hour wetted period per irrigation. This suggested that if the required amount of water could be applied in a shorter time perhaps sprinklers could be used without danger. The factors seemed to be the length of the high humidity period necessary for successful infection and the time required for the bacteria to ooze out of infected tissue for dissemination to adjacent plants.

*Materials and Methods.*—To test the above hypothesis a field experiment with common bacterial blight was designed to provide irrigation periods of 3, 6, 9, and 12 hours. A 2-acre field was planted uniformly with Red Mexican beans in 2-ft. rows and irrigated initially by furrows. A portable sprinkler system was used as in the previous experiment with sprinkler locations set on an 80 ft. × 80 ft. pattern. Each sprinkler location was considered to be a plot. When the bean plants were about 6 in. high a strip of plants 6 ft. long adjacent to each sprinkler setting was inoculated with a virulent culture of the bean blight organism. Inoculation was successful at all locations.

The sprinkler system was available for only 2 periods of differential application. These were made on July 12 and August 2. The 12-hour treatment was started at 7:15 a.m. with all other sprinklers cut off. The other treatments were begun at 3-hour intervals so that all could be terminated at 7:15 p.m. In this way

the duration of high humidity after treatment would be the same for all treatments.

Because the sprinklers were spaced too far apart to irrigate all plants in the field, supplemental furrow irrigations were made on the entire field as needed. This meant that the beans sprinkled for the longer periods received more total water but all were irrigated adequately and drainage was good enough that none received damaging amounts. No attempt was made in this experiment to equalize the total amount of water applied by the different sprinkler schedules since wetted time rather than amount of water was believed to be the significant factor.

*Results.*—At the time of the first sprinkler application, general infection was present on plants within the inoculation sites beside each sprinkler. Throughout the rest of the season occasional secondary infections were found on the inoculated plants but no infections were found on plants outside the inoculation site regardless of irrigation treatment. Plants were maturing by August 16 when final observations were made.

EFFECT OF DURATION AND RATE OF SPRINKLER DELIVERY ON BLIGHT SPREAD.—In an effort to explain the results of the above experiments a third trial was conducted in 1952. Three sprinkler lines were laid out on a uniform stand of Red Mexican beans, each line with 6 sprinklers set at 40-ft. intervals. Each sprinkler was equipped with a shut-off valve and a nozzle size selected so that approximately equal amounts of water would be added in 8, 12, 18, 24 and 30 hours. This was approximated by using nozzles of 7/32 in., 11/64 in., 9/64 in., 1/8 in. and 7/64 in. bore. A sixth 4-hour treatment with the 7/32 in. nozzle completed the series.

Because of a possibility that the inoculation locations used in 1951 were too close to the sprinkler, 4 centers were inoculated around each sprinkler, 2 located 7 ft. from the sprinkler and 2 placed 14 ft. from the sprinkler. Since each sprinkler was adjusted to wet a circle 25 to 30 ft. in radius, these locations tested both the fine spray near the center and the coarser droplets towards the outer edge.

The plots were inoculated when the plants were about 6 in. high with 3-day-old beef-broth cultures of the blight bacteria. The inoculum was diluted 1:10 with water and applied with a 3-gal. portable sprayer. The plants in each inoculation center (4 ft. of one row) were enclosed in a paper barrier and lightly injured with a steel brush before inoculation. Inoculations were made in the evening of July 18 after a 3-hour sprinkling. Heavy initial infection, ultimately resulting in almost complete defoliation, was obtained in all inoculated centers.

Three differential irrigations were applied on August 5, 14, and 26. By the end of August the beans were maturing so no further tests were possible. The individual sprinklers were started for each test at intervals starting at 10:00 a.m. so all could be turned off the following day at 4:00 p.m.

*Results.*—During the course of the experiment the initial inoculation centers were badly defoliated by blight but no obvious spread of infection to adjacent rows was noted. Ten days after the last irrigation detailed notes were taken on disease spread associated with each infection center. The results can be summarized as follows:

4-hour wet periods. A few secondary infections developed within inoculation centers but only a trace of infection on plants in the next row.

8-hour wet periods. Same result.

12-hour wet periods. Secondary infections in inoculation centers more numerous than in the 2 previous treatments, but spread to adjacent rows still only a trace.

18-hour wet period: Same result.

24-hour wet period: Same result.

30-hour wet period: Same result, except that in 1 replicate there was slightly more spread to adjacent row.

With all treatments the damage from blight in the inoculation centers appeared to be severe but in spite of leaf damage and defoliation there was little pod infection. At harvest time 12 representative yield samples were taken from the inoculation centers of 1 replicate along with comparable check samples from adjacent rows. The inoculated plants yielded at the rate of 24.6 cwt. per acre compared to 27.5 for the healthy checks. These yields are normal and the differences were not statistically significant.

GREENHOUSE EXPERIMENTS.—Following the failure to obtain spread of blight with sprinklers in the 1951 field test, greenhouse experiments were performed to determine how long inoculated plants need to be kept at high humidity to insure infection. Zaumeyer[3] found that infection readily occurred when plants were kept in a saturated atmosphere for 24 hours before and after inoculation. If the pre-inoculation humidity treatment was omitted infection was still heavy, but when the plants were inoculated without any high humidity treatment infection was very slight. He did not report results on shorter moist periods or pre-inoculation high humidity only.

Red Mexican beans, 10 plants per 6-in. pot, were inoculated when 8 in. high and in a very succulent condition. Inoculum was prepared by diluting 48-hour-old beef broth cultures of *X. phaseoli* in distilled water in a ratio of 1:2. The inoculum, to which carborundum powder was added, was applied to the plants with a small hand-operated atomizer sprayer. Humidity treatments and results are in Table 1. Although the heaviest infection occurred on the plants kept in the moist chamber for 24 hours, there was considerable infection even on plants left to dry off quickly on the greenhouse bench.

A second experiment was made on Red Mexican bean growing in flats. These were tested when 8 in. high and in a very succulent condition. The inoculum was prepared in the same way as before except that

[3] Zaumeyer, W. J. 1930. The bacterial blight of beans caused by Bacterium phaseoli. U. S. Dept. Agr. Tech. Bul. 186.

TABLE 1.—*Effect of length of high humidity treatment after inoculation with X. phaseoli on number of lesions produced on Red Mexican beans*

| Length of high humidity period after inoculation | Number of lesions on third node leaves from 10 plants | | | | |
|---|---|---|---|---|---|
| | Rep. 1 | Rep. 2 | Rep. 3 | Rep. 4 | Average |
| (hrs.) | | | | | |
| 0 | 29 | 59 | 13 | 16 | 29 |
| 3 | 45 | 25 | 26 | 25 | 30 |
| 7 | 32 | 65 | 29 | 39 | 41 |
| 12 | 23 | 18 | 70 | 26 | 34 |
| 20 | 40 | 35 | — | — | 38 |
| 24 | 66 | 66 | — | — | 66 |

the dilution was 1:10. Results were recorded as the number of plants having lesions on the third node leaf which was the youngest fully expanded leaf at the time of inoculation.

Treatments and results were as follows:

a) Inoculum applied as a fine spray on dry plants with no subsequent high humidity treatment—3/35 plants infected.

b) Inoculum applied as a fine spray on dry plants followed by 24-hour high humidity treatment—2/31 plants infected.

c) Same as (a) but plants thoroughly wetted first. No subsequent high humidity treatment—18/27 plants infected.

d) Same as (b) but plants also thoroughly wetted first—31/33 plants infected.

DISCUSSION.—The greenhouse inoculation tests demonstrated that bean plants can be infected with the bacterial bean blight organism without a 24-hour high humidity period afterwards. A thorough wetting of the plants prior to or during inoculation is all that is necessary for infection. These results are supported by the field inoculations which were uniformly successful even though the plants were allowed to dry after inoculation in the 1951 and 1952 tests.

In view of these results the failure of 30 hours continuous sprinkling to effect more than a trace of blight spread from heavily infected plants cannot be attributed to lack of sufficient humidity. It seems more likely that the field results can be explained best on the basis of temperature rather than moisture. In 1950 bacterial blight inoculation was successfully carried out on July 18. But secondary spread did not become evident until early September after which the disease spread rapidly. The 1951 and 1952 tests were conducted in July and August and, because of earlier planting, the plants matured before cooler fall weather occurred.

Since primary infections were readily obtained during hot weather the most plausible explanation of the failure of secondary infections is that viable inoculum did not survive between sprinklings in high enough concentration. A low level of inoculum only sufficient to carry the disease through until fall was obtained.

This explanation is in accord with all field observations on bacterial bean diseases in this area. Common blight and, to a lesser extent, bacterial wilt have often been observed on sprinkled beans late in the season. Neither of these diseases has been observed to a significant extent before September. Thus, in the area under discussion, these diseases have only become obvious near harvest time when maturation of vines is desirable.

During only 1 season since any of these bean diseases were recorded in central Washington have they been observed on non-sprinkled beans. This was in the summer of 1947, a season characterized by unusual frequency of summer rainfall sometimes accompanied by hail. That year a trace to light infection of bacterial blight, halo blight, and bacterial leafspot was recorded in the Moses Lake area on experimental plantings of snap beans and lima beans.

It seems safe to conclude that in a hot, arid climate such as prevails in the Columbia Basin during the growing season, sprinkler irrigation will not favor bacterial diseases of beans to the extent that significant damage is incurred. There may be occasional years, such as 1947, when unusual summer rains with accompanying lower temperatures could lead to bacterial disease losses in sprinkled beans. Rainfall records taken at the Irrigation Experiment Station, Prosser, show that 1.52 in. of rain fell during July and August, 1947, the highest recorded in 30 years. The wettest July and August since then was 1953 when 0.74 in. of rain fell without favoring unusual outbreaks of blight in sprinkled beans. This figure has been exceeded only 3 times in the 30 years of weather records at this station.

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# THE PATHOLOGY OF ELYTRODERMA DEFORMANS ON PONDEROSA PINE[1]

Paul C. Lightle[2]

SUMMARY

*Elytroderma deformans* (Weir) Darker, the cause of a witch's broom of ponderosa and Jeffrey pines and a needle cast of these and several other species, has become increasingly destructive during the past few years.

Studies designed to augment our knowledge of the biology of the fungus showed that: 1) mild temperature and high moisture are important factors in ascospore discharge and that these 2 occurred together in the fall of 1950; 2) culture media ordinarily used for growing fungi were inadequate for growing *Elytroderma;* and 3) several organic and inorganic materials that are known to promote germination of spores of other fungi did not induce the germination of ascospores of *Elytroderma.*

Investigation of the host-parasite relationship revealed: 1) The fungus does not penetrate the shoot through the leaf, but apparently lives entirely within the needle. 2) In the needle, the fungal hyphae are confined to the cells of the mesophyll and the lacunae of the resin canals. 3) Cells of the phloem tissue are caused to degenerate by an unknown substance, which is thought to be a phytotoxin produced only when the fungus is present. The degeneration of the phloem is not confined to the affected needles, but extends through the short shoots, and for an undetermined distance proximally in the twig. 4) Groups of degenerating phloem cells provide, in many cases, the stimulus for pathological resin cyst formation. These are located in the bark in the peripheral zone of phloem and, after the second year, may be clearly seen with the unaided eye.

*Elytroderma deformans* (Weir) Darker is an Ascomycetous fungus causing defoliation of, and other injury to, several species of pines. Ponderosa (*Pinus ponderosa* Laws.) and Jeffrey (*P. jeffreyi* Murr.) pines, in particular, are severely injured and often prematurely killed.

A member of the family Hypodermataceae, the fungus was first named *Hypoderma deformans* by Weir (15) in 1916. Darker (3), in his monograph on the taxonomy of the members of this group living on conifers, considered the large 2-celled ascopsores of the species sufficiently distinctive to set it apart as a new monotypic genus. He also considered that Weir's investigation of the fungus had included the only really satisfactory infection experiments with any of the Hypodermataceae.

In addition to describing *Elytroderma deformans* and performing infection experiments with it, Weir made spore germination tests, using ascospores, and attempted to grow the organism in pure culture. He made observations on the distribution and abundance of the disease and determined the general course of its life cycle. No other detailed work on this species has been noted in the literature.

The disease has become increasingly prevalent during the past 5 years and the destructive effects of repeated heavy attacks are very apparent. Clarification of some of the previous work and additional information on the biology and life cycle of the fungus have been needed in order to develop control measures and to forecast more accurately the occurrence and severity of attacks. The purpose of the present paper is to report the results of investigations toward these ends.

PREVALENCE AND DAMAGE.—Elytroderma needle cast is essentially a disease of western pines. It has been reported from British Columbia south through Washington, Oregon, California, Montana, Idaho, and Colorado in the West, from Lake Timagami in Ontario, and from Elijay, Gilpin County, Georgia (3). Ellis (4) found the disease widespread in 3 national forests in Arizona, and Gill[3] states that the disease is common in New Mexico.

Field studies in California and Oregon during 1949 and 1950 showed that the fungus attacks trees of all sizes, from seedlings 6 in. high to mature trees. The youngest infected tree found, with the presence of dead needles bearing hysterothecia as a criterion, was determined to be 11 years old; only 1 such plant was found. Infected trees 12 years old were fairly common, but infection was more abundant in trees 14 years old and older.

The disease is most common on young trees in stands

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The author is also indebted to the members of the U. S. Forest Service on the Ochoco and Boise National Forests in Oregon and Idaho, respectively, for their cooperation in making periodic collections of diseased needles, and particularly to Ray W. Ellstrom and Orville B. Cary, who set out and changed spore traps during the period from September 25, 1950, to August 13, 1951.

[3] Memorandum from Lake S. Gill, Senior Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, dated November 8, 1948.

of reproduction up to 20 ft. high. This may be due to the relatively large number of plants of this size. Young plants are seldom killed but may be badly deformed. Extensive killing of saplings has been observed in a few localities, principally, but not entirely, in overstocked stands. In Oregon, older trees of sawtimber size are frequently killed, whereas in California such trees are usually only deformed. No reason for this difference was found, but differences in climate are suspected of being responsible. In Oregon the normally mild temperatures and frequent rains during May and June are thought to be conducive to attacks by secondary organisms, which cause the death of the tree before it can recover from the shock of losing a major portion of its needles. Starvation from repeated defoliation is undoubtedly a major factor and cannot be discounted.

The pattern of the disease under natural conditions indicates that attacks are heaviest at higher altitudes and damage is most severe in ravine bottoms and least on the ridges. The same situation was noted by Wagener et al (14).

The disease has been found only on pines, including *Pinus banksiana* Lamb., *P. contorta* Dougl., *P. echinata* Mill., *P. edulis* Engelm., *P. jeffreyi* Murr., and *P. ponderosa* Laws. (3).

Economic losses have never been estimated, but Wagener et al (14) reported that the disease developed in severe form on *Pinus ponderosa* in many localities east of the Cascade Mountains during 1946, 1947, and 1948. During the 2 latter years it was almost equally severe in some localities in southern Idaho. Other areas of heavy infection of less extent developed on the Salmon National Forest in Idaho and in Lassen Volcanic National Park and Latour State Forest in northern California. Pine foliage in entire basins was turned a straw yellow to deep red in color, with the exception of small portions of the tops.

During recent years extensive logging operations have been necessary to salvage infected pines in the Ochoco and Whitman National Forests in central Oregon and in the Boise and Payette National Forests in southern Idaho. Wagener et al (14) reported approximately 90 per cent mortality of ponderosa pine in the worst infected areas on the Ochoco National Forest. There, salvage cutting between 1946 and 1952, to utilize dead and dying trees, amounted to over 98,148,000 board feet.[4] Approximately 70 per cent of this was timber that would otherwise have been lost. Comparable losses have occurred on the Whitman National Forest and in southern Idaho.

SIGNS AND SYMPTOMS.—The early stages of Elytroderma needle blight are extremely difficult to distinguish, because they so closely resemble the early stages of infection by other needle-cast fungi, notably *Hypodermella medusa* Dearness, and by nonparasitic disorders, the most common being winter injury. Such early manifestations of disease as needle tip kill and spot kill on some of the needles are indicative of infection by *Elytroderma deformans*, but may be due to a variety of other causes.

Weir (15) reported great irregularity in the time between the first browning of the needles and the appearance of mature apothecia but in no instance were infected needles of trees not previously attacked by the fungus killed before they had attained normal size. He indicated that the needles usually turned brown in September or October. The first signs of the apothecia were noted during the late fall, and mature spores were found the following spring.

The developmental course of the disease described by Weir does not coincide with that occurring in the west wherever observed over appreciable periods. The present study indicates that development is as follows:

In late fall the infected green needles of the current season begin to fade. Frequently, only the distal portion from a point approximately midway is involved at this time. By January or February the infected needles, from the tips to the sheaths, are definitely lighter in color than similar areas on noninfected needles. As spring approaches, affected leaves take on a distinctive reddish cast, which is most pronounced during May and early June, and which, until hysterothecial development begins, might be confused with coloration caused by winter injury. The portion of the needles near the distal end of the sheath often remains alive and green for some time. As the new green leaves make their appearance, the reddish color of the diseased needles gradually fades until the normal straw color of dead needles obtains.

During May or early June the imperfect stage is formed. The pycnidia are concolorous with the leaf and appear as small blisters about 1 mm. long. Under proper conditions of moisture the pycnidiospores are exuded in tendrils, which usually aggregate into tiny, somewhat spherical droplets. During dry weather these soon assume a granular appearance en masse, but are quickly dissolved and washed away by rain. The granular spore masses may be seen readily with the aid of a hand lens and are also visible, upon close inspection, with the naked eye.

The perfect stage of the fungus usually begins to form on the dead portion of the needle during the latter part of June.[5] The hysterothecia are first evident as slightly brownish or purplish lines 1–2 mm. long. These gradually darken and lengthen until at maturity they appear black and shiny and average 10 mm. in length. Such lines of hysterothecia are the most definitive signs of the disease. Needles killed by the disease are usually shed late the following fall or early the next winter.

In addition to needle manifestations, other symptoms are evident in certain diseased twigs and branches. The

[4] Memorandum from the Supervisor, Ochoco National Forest, Prineville, Oregon, dated March 23, 1951.

[5] T. W. Childs, Pathologist, Division of Forest Pathology, U. S. Dept. of Agriculture, stated, in a letter dated March 27, 1952, to Paul C. Lightle, that he has 2 collections of June 8—one in 1949 and the other in 1950—in which the hysterothecia are faintly visible to the naked eye.

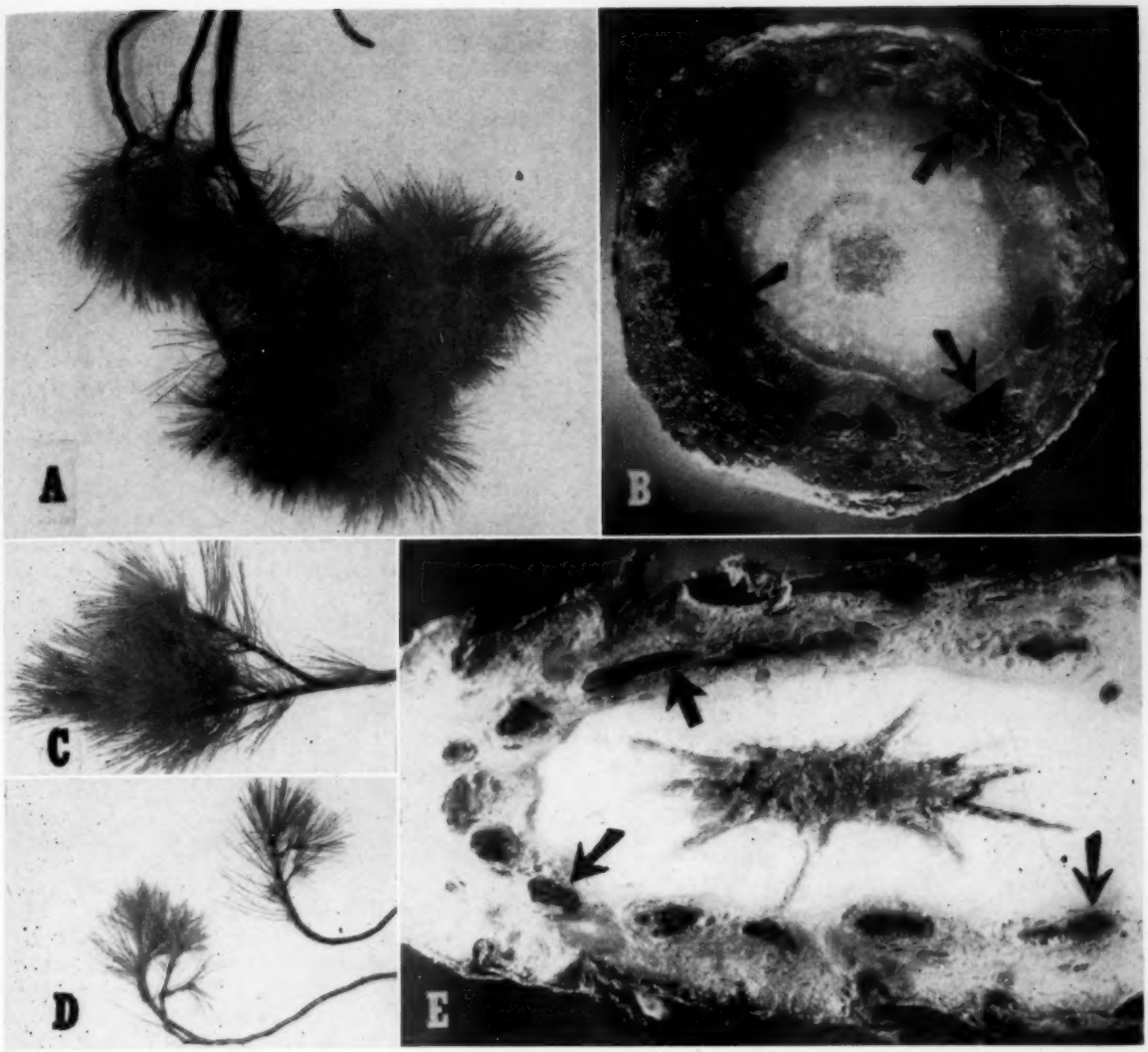


FIG. 1. Effects of *Elytroderma deformans* on branches of *Pinus ponderosa*. A) Large, vigorous witch's broom. B) Pathological resin cysts shown in transverse cut. C) Normal twig. D) Dwarfed branches. Note the upward curving of these twigs and their short, sparse needles. E) Pathological resin cysts shown in oblique cut. Note the proximity of the resin cysts to the zone of phloem in Fig. B and E.

most striking of these is an abnormality termed a witch's broom, which is produced at the ends of scattered branches in the crown of ponderosa and Jeffrey pines. The brooms vary in size, from small balls 4–6 in. in diameter, consisting of densely packed needles of nearly normal length, to larger ones several feet in diameter and of the same or of a somewhat more open character (Fig. 1, A). The weight of the latter usually causes them to hang pendant-fashion, but this condition is not invariable. The branches supporting such abnormalities are somewhat enlarged in comparison with normal branches, but the large size of the brooms often makes the branch parts appear attenuated. Brooming appears to be confined to thrifty trees where only occasional branches are heavily infected. This may possibly be an expression of resistance by the tree, but as yet the facts are not sufficient to warrant such a conclusion.

Trees with the major portion of their crowns heavily infected with *Elytroderma deformans* and trees of reduced vigor usually do not form witch's brooms. Instead, there appears to be a dwarfing effect (Fig. 1, C and D) characterized by some thickening and often an abnormal curvature upward of the twigs, a reduction in the number of needles, and a shortening in length of both twigs and needles. In some cases the latter are less than 1/3 normal length. There is also a stimulation of lateral buds with the formation of only a relatively few needles on each bud. Such branches are not pendant, but maintain a normal position. Dwarfing is by far the most common effect noted in California and Oregon.

Needle infection does not necessarily lead to twig deformation. Trees in areas heavily affected for a single season have been observed to recover completely the following year with no visible effects other than a reduction of lateral growth for a season.

An additional sign of the disease is the presence of resin cysts in the peripheral part of the phloem zone of the bark (Fig. 1, B and E) of twigs and smaller branches. Slicing through the bark tangentially, transversely, or obliquely shows these structures to be fairly numerous, 0.5–5.0 cm. or more in length, and 1–5 mm. in cross-sectional dimensions. These areas are not usually seen in growth less than 3 years old, but a few have been observed in 2-year-old tissue. They are not found in healthy twigs.

BIOLOGY.—*Fruit body and spore formation.*—Samples of diseased pine needles were collected at irregular intervals during the years 1949, 1950, and 1951. The stage of development of ascospores was determined by examining them under a microscope. Dead needles without hysterothecia, but suspected of being infected with *E. deformans*, were examined under a low-power microscope to ascertain the presence of pycnidia and the occurrence of spore exudate. This gave a fairly accurate picture of the variation in the development of fruiting bodies and spores for different seasons, different regions, and different samples from the same region. However, the spore tendrils are easily dislodged from the needles and are readily dissolved in rain or dew, and so their absence does not necessarily mean that they were not produced.

Table 1 presents data obtained from these systematic observations on the development of pycnidia and hysterothecia. It indicates that pycnidia are produced quite generally prior to early July and that some evidence of spore tendrils may be found during most of the year. No ascospores were found in July or August, but were found in all stages of development in September. By the end of October, nearly all ascospores noted were mature as to size and the presence of a septum. Whether or not they were physiologically mature was not determined. Most infected needles were shed before December in 1949, but were retained until January during the winter of 1950-1951.

*Spore discharge.*—The granular exudate, consisting of large numbers of pycnidiospores, and the thin tendrils of freshly extruded spores are both readily dissolved in water and the spores can thus be spread

TABLE 1.—*Development of the imperfect and perfect stages of Elytroderma deformans (Weir) Darker during 1949 to 1951*

| Collection Data | | | Imperfect stage | | Perfect stage | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Ascospores | | | |
| Area[a] | Month and Year | No. places collected | Relative abundance | Spore tendrils | Relative abundance | Immature | Mature | Germinated | Remarks |
| | July | | | | | | | | |
| Northern | 1949 | 2 | ++[b] | 0 | ++ | * | 0 | 0 | Not yet formed. |
| Central | 1949 | 1 | ++ | 0 | +* | 0 | 0 | 0 | Just starting. |
| Southern | Aug. '50 | 1 | + | 0 | + | * | 0 | 0 | Not yet formed. |
| | Sept. | | | | | | | | |
| Northern | 1950 | 1 | + | 0 | +++ | + | +++ | 0 | |
| Central | 1949 | 3 | ++ | + | +++ | +++* | 0 | 0 | Some spores non-septate. |
| | Oct. | | | | | | | | |
| Northern | 1949-50 | 5 | + | + | +++ | ++ | +++ | + | |
| Central | 1949 | 2 | + | 0 | ++ | +* | +++ | + | |
| Southern | 1949 | 1 | ++ | + | +++ | + | +++ | 0 | Some spores aborted. |
| | Nov. | | | | | | | | |
| Northern | 1950 | 1 | + | + | +++ | 0 | +++ | ++ | '48 needles shed; '49 dy- |
| Central | 1949 | 2 | 0* | 0 | + | 0 | ++ | + | ing but no fruiting. |
| | Dec. | | | | | | | | |
| Northern | 1949-50 | 2 | + | + | +++ | 0 | +++* | + | Many asci empty. |
| Central | 1949 | 1 | 0* | 0 | 0 | 0 | 0 | 0 | '48 needles shed; '49 dy- ing but no fruiting. |
| Northern | Jan. '51 | 1 | 0 | 0 | 0* | 0 | 0 | 0 | |
| | Feb. '51 | 1 | + | + | ++ | 0 | ++* | + | '49 needles shed; '50 dy- |
| | Mar. '50-51 | 2 | 0 | 0 | + | 0 | +* | 0 | ing but no fruiting. |
| | Apr. '51 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | Most asci empty. |
| | May '50 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | Most infected needles shed. |
| | June '50 | 1 | +++ | + | 0 | 0 | 0 | 0 | '49 needles red. |

[a] The Northern area includes Oregon and Idaho; the Central area includes northern California north of Yosemite N. P.; the Southern area is all of Southern California.

[b] Key to symbols used in Table 1:
0 = Lacking; + = Sparse; ++ = Moderate; +++ = Numerous; * = See notes under remarks.

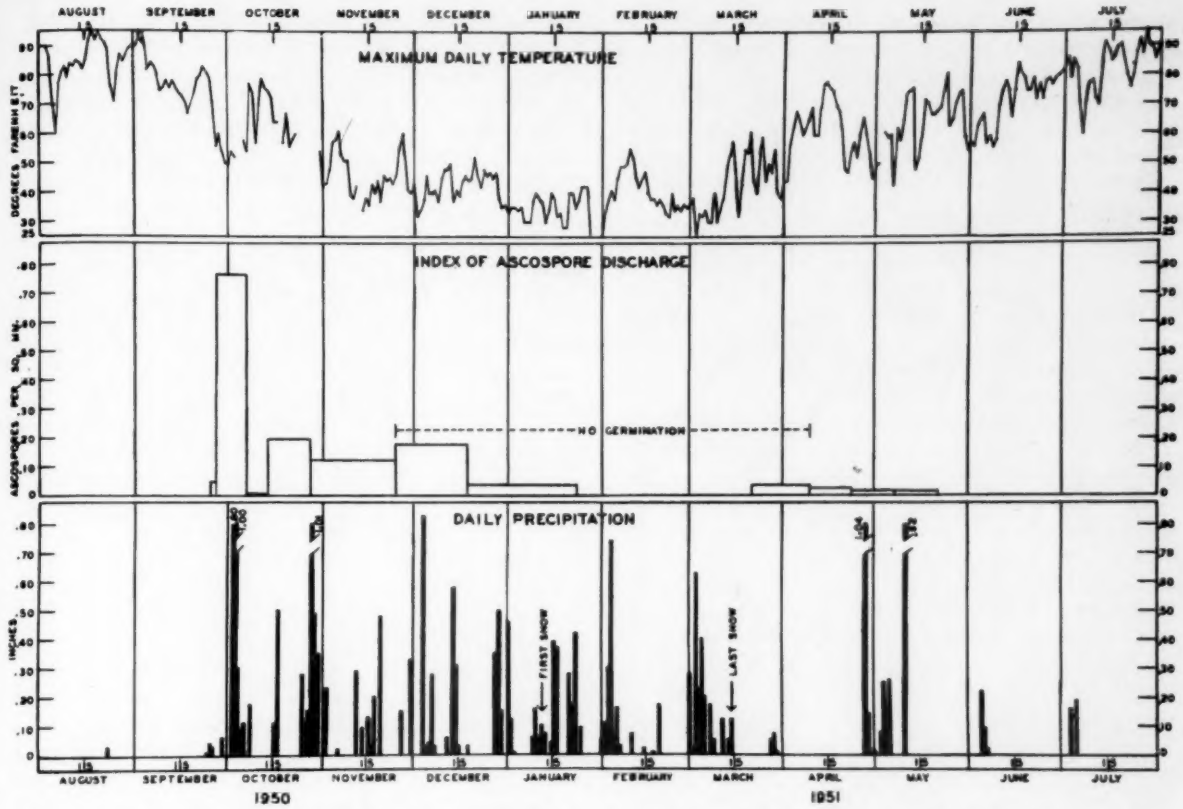


FIG. 2. Ascospore discharge in relation to temperature and precipitation. The center graph shows the number of ascospores deposited, per sq. mm. of slide exposed, for the period of days indicated by the bar.

easily by the spattering or washing action of raindrops.

According to Weir (15), the ascospores of *E. deformans* do not all mature at the same time. He stated that they are forcibly discharged, being shot out from the asci a distance of 1–2 mm., and can be found at any season. Presumably, the spores are caught, after discharge, by air currents and carried off to infect other plants or to reinfect the plant they came from. They might also be spread in droplets of moisture during rain or fog.

During the fall and winter of 1950-1951, spore traps were placed in various areas of the Ochoco National Forest in an attempt to determine the time of year when ascospore discharge is heaviest and the correlation, if any, of spore discharge with seasonal and climatic conditions. Spore traps consisted of clean microscope slides, labeled and coated with a thin layer of vaseline petroleum jelly. They were exposed for varying numbers of days in ponderosa pine stands, in which the disease was prevalent, in the following situations: 1) in a clearing with the closest trees 30 ft. away; 2) in a small opening with the closest trees 10 ft. away; 3) under a log with the closest trees 2 ft. away; 4) directly under a limb of an infected tree; 5) in an opening with the closest trees 40 ft. away; 6) in thickets of reproduction, including saplings.

In each case the traps were protected from the direct force of the elements by placing them under a board, log, or other shelter, keeping at least 12 in. of air space between.

From September 26 to November 24, 1950, the spore traps were exposed at 2 locations about 3 mi. apart. From November 24 until the close of the experiment, the slides were all located in the same general area. Heaviest ascospore discharge was found to occur in late September and October during and following the first fall rains. At this time, both moisture and temperature were conducive to spore discharge (Fig. 2). Variations in relative abundance of ascospores caught are shown graphically in Fig. 2. The index represents a minimum rather than a maximum measure of probable abundance, because in examining the traps only ascospores that could be recognized with certainty as those of *E. deformans* were counted. The graph does not show the fact that a high percentage of the spores caught from September 25 to October 27 either germinated on the slide or were germinated when caught. The number of germinated spores found on the slides decreased as the year waned. None were found between November 24, 1950, and April 9, 1951. Only a very few were noted between April 9 and May 7.

*Spore germination.*—Spore germination tests were attempted, with both ascospores and pycnidiospores. For the ascospore tests, various substances were em-

ployed which are known to promote germination of spores of other fungi, and the tests were replicated at least twice for each substance. A drop of each, in water solution, was placed on a flamed microscope slide and ascospores were added. Such slides were placed in petri dish moist chambers in pairs and examined under a microscope every 24–48 hours and the germination was recorded.

The pycnidiospores were tested, in duplicate, only in distilled water by the hanging drop method or by placing a large number of spores in 5 ml. distilled water in a test tube and examining each day for 7 days.

All germination tests except one were conducted at an elevation of about 200 ft.; the one was made at an elevation of approximately 5500 ft.

Results of the ascospore germination tests are in Table 2. The large size of the spores (90–118 × 6–8$\mu$), and the fact that they germinate from either or both of the cells, very nearly excludes the possibility that germination occurred but was not detected. Spores in a moist, nearly saturated atmosphere were plasmolyzed and it was necessary to add sucrose to bring the concentration of the test material to 2 per cent to prevent them from bursting. Perhaps this was due to the insulating effect of the gelatinous sheath (6–10$\mu$ in thickness) around each spore. None of the materials tested gave much indication of improving germination. Vitamin $B_1$ caused a warty appearance of the spores, and Vitamin $B_2$ seemed to be responsible for the spores breaking apart at the septum. The only germination noted occurred in concentrations of 2, 3, and 4 per cent sucrose.

Only once during the course of the experiments was good germination obtained. This occurred the last week in September 1951, at an elevation of 5500 ft. ±. Ascospores were placed in a 2 per cent sucrose solution, as for the other tests, and held at 60° to 70° F. for 24 hours. At the end of this period nearly every spore observed had germinated. Two weeks later the experiment was repeated at 200 ft. ± elevation, under a bell jar that had been evacuated to approximate the 5500 ft. elevation. The results were completely negative. The reasons for this have not been determined, but investigations are continuing.[6]

Pycnidiospores placed in a test tube of distilled water showed some germination after 3 days. The first indication of budding was a warty appearance of the spores. Six hours later, germ tube formation had started and a few spores with tubes up to 3 $\mu$ in length were found. These, however, did not elongate further.

Some of the hanging drop preparations of pycnidiospores were sealed to the slides with vaseline and others were kept in moist chambers. There was no germination of spores in the sealed slides and only 2 spores on 1 of the unsealed slides were germinated on the fourth day. One of these put out a short secondary branch after growing to a length of 7 $\mu$. The other remained unbranched, growth ceasing after reaching a length of 2.5 $\mu$ (Fig. 3, A).

*Culture studies.*—Weir (15) reported that he was able to germinate the spores of *Elytroderma deformans* readily and that in some cases an abundant white mycelium was produced, but ". . . in the course of 6 months, after frequent transfers, the mycelium turned a light yellow and died." Attempts were made to obtain *E. deformans* in culture by employing different source

[6] In recent unpublished tests with spore material from Idaho, James L. Mielke of the Logan, Utah, laboratory of this Division, obtained abundant germination of ascospores at temperatures of 10°–15° C., indicating that the temperature required for germination of this spore form of the fungus is relatively low.

TABLE 2.—*Results of germination trials with ascospores[a] of Elytroderma deformans (Weir) Darker*

| No. Trials | Medium | Concentrations per cent | | | | Temperature | Results |
|---|---|---|---|---|---|---|---|
| 2 | Moist air from moist chamber | ---- | ---- | ---- | --- | Room | Spores Plasmolyzed—no germination. |
| 4 | Distilled $H_2O$ | ---- | ---- | ---- | --- | 2 at Room 2 at 34°C. | Many spores burst—no germination. |
| 12 | Sucrose | 1.0 | 2.0 | 3.0 | 4.0 | 4 of 2 per cent at 34°C. Others at Room | Few short germ tubes produced in 2, 3, 4 per cent at Room temperature. No other germination. |
| 6 | Glycene[b] | 0.1 | 0.3 | 0.6 | --- | Room | No germination. |
| 6 | Galactose[b] | 1.0 | 3.0 | 6.0 | --- | Room | No germination. |
| 6 | $K_2HPO_4$[b] | 0.1 | 0.3 | 0.5 | --- | Room | No germination. |
| 6 | Vitamin $B_1$[b] | 0.1 | 0.003 | 0.001 | --- | Room | Some spores turned rough and warty in appearance after 5 days. No germination. |
| 6 | Vitamin $B_2$[b] | 0.1 | 0.003 | 0.001 | --- | Room | Some spores seemed to break apart at septum. No germination. |
| 6 | K Mn $O_4$ | 0.01 | 0.03 | 0.10 | --- | Room | No germination. |

[a] Ascospores were obtained from hysterothecia on Jeffrey pine needles collected on October 11, 1949, and the above trials were run between November 11 and December 12, 1949.

[b] Two per cent sucrose was added to each concentration to prevent the spores from bursting.

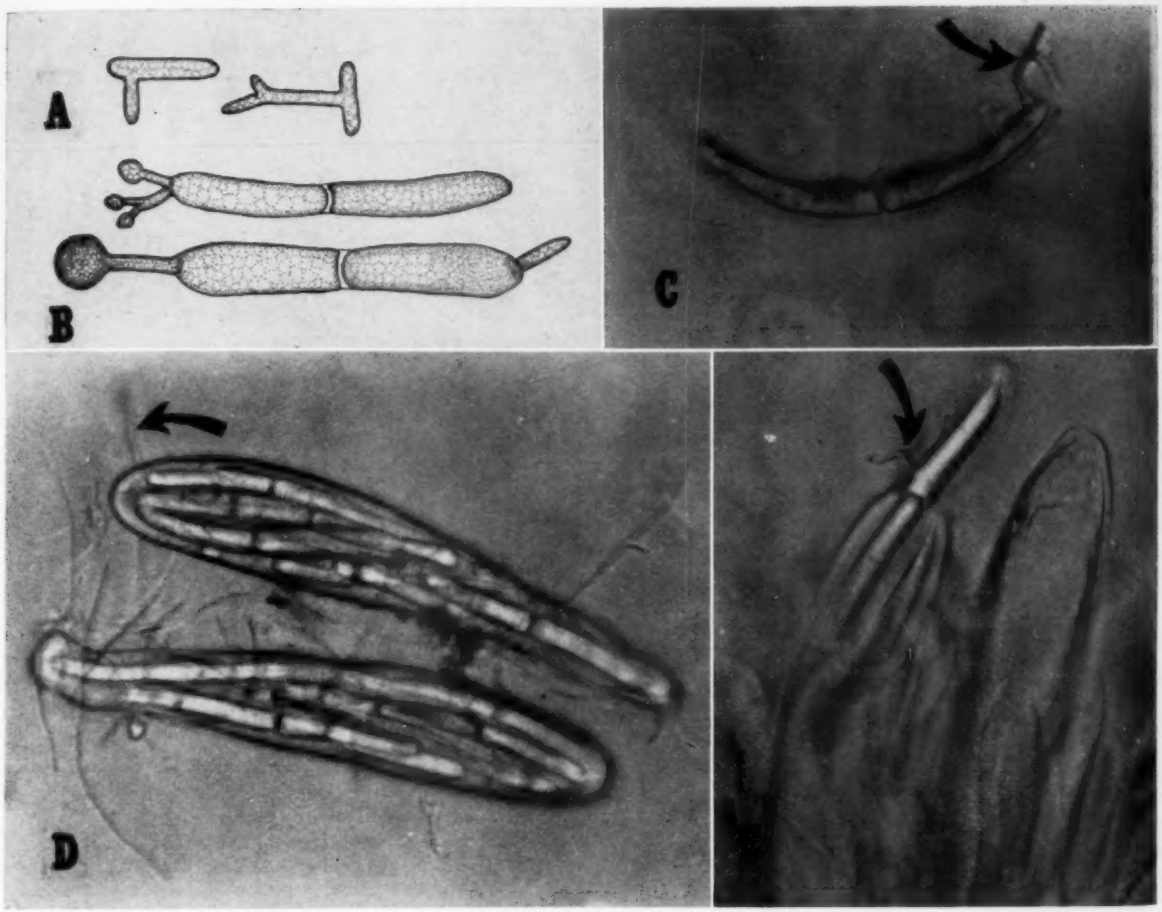


FIG. 3. Germination of *Elytroderma deformans* spores. A) Pycnidiospores (about 2000×). The germ tube on the spore at the left is 2.5μ long. The branch in the germ tube on the spore at the right occurred at 7μ. B) Ascospores (about 400×). Note the short germ tubes produced by both cells of the lower spore and the rounding off of the tips, which apparently is induced by too high temperatures. C) Germination of ascospore showing a branching germ tube (about 500×). D) Germination within the ascus. The ascus at the bottom contains ungerminated spores, whereas the upper one has a germ tube, from a spore near the tip, protruding through the side of the ascus. E) Germination within the hysterothecium. The partially discharged spore has a germ tube at the left near the septum, and the gelatinous sheath can be seen around the free end of the spore. The ascus on the right is empty. (D and E about 400×).

materials, different culture media, and by incubating the cultures at different temperature levels. Each specific combination of source material, medium, and temperature was replicated many times in the course of the trials.

In the experiments with ascospores as source material, 2 methods of obtaining the spores and introducing them onto the plates were used:

1) Hysterothecia-bearing needles were placed in distilled water and allowed to discharge spores for 2–3 hours. These were then streaked on a plate with a platinum loop needle, or 1 ml. of spore suspension was withdrawn and transferred to the agar and spread by tilting the dish. This method resulted in an extremely high number of contaminants. Dipping the needles in alcohol and then flaming did not reduce contamination to a satisfactory level.

2) A pine needle containing mature hysterothecia was laid on a glass slide and water added to induce the fruit bodies to open. The contents were then removed with a dissecting needle and teased apart. Ascospores were transferred to pieces of pine needle embedded in agar, or the entire mass was washed to the surface of an agar plate and dispersed by tilting the plate at various angles.

Small pieces of pine needles containing hysterothecia were placed directly on the surface of the agar, but again contamination was so heavy, even after surface sterilization, that trials were discontinued.

Pycnidiospores were prepared in distilled water as previously described and were then streaked over the surface of an agar plate.

Attempts to obtain *Elytroderma deformans* in culture resulted in complete failure. Table 3 summarizes the many variations of culture media, inoculum, and temperature employed. In some trials with ascospores, a few spores germinated and produced short germ

TABLE 3.—*Results of attempts to culture Elytroderma deformans (Weir) Darker*[a]

| No. Trials | Medium[b] | Source Material[c] | Method | Incubation temperature | Remarks |
|---|---|---|---|---|---|
| 27 | I | 12 of 1<br>15 of 2 | Spotted on surface | Room | All contaminated. |
| 4 | II | 3 | Poured on surface | Room | No growth. |
| 8 | III | 3 | 1 to 10 and 1 to 100 dilutions poured on surface | Room | No growth. |
| 8 | III | 5 | 10 ml. on surface for 20 minutes, then poured off | Room | No growth. |
| 12 | III | 4 and 6 | 3 poured on surface<br>9 streaked on surface | Room | No growth. |
| 2 | III | 3 | Streaked on surface | Room | Some spore germination; no growth. |
| 1 | III | 1 presoaked for 2 hours | Spotted on surface | Room | No growth. |
| 4 | II | 6 | Poured on surface | Room | No growth. |
| 34 | II, IIA, IV, IVA, IV + gas-sterilized needles | Single spores from above | Germinated spores from above spotted on surface | Room | No growth. |
| 12 | I, IV | 8 | Poured on surface | 2 each at 13°, 16°, 19°C. | No growth. |
| 24 | I, II, IV, IX | 7 | Natural fall | Room, 13°, 16°, 19°C. | No growth. |
| 12 | I, IV | Single spores from above | Spotted on surface | 2 each at 13°, 16°, 19°C. | No growth. |
| 31 | V, VI, VII | 16 of 8<br>15 of 3 | Poured on surface | 2 each at 5°, 13°, 16°, 19°C. | No growth. |
| 2 | II | 9 | Streaked on surface | Room | No growth. |
| 10 | VIII | 10 | Spotted on needles | Room | No growth. |

[a] The material from which the hysterothecia and the ascospores were obtained was collected on October 28, 1948, and on March 30, 1949. The material containing pycnidiospores was collected on July 27, 1949.

[b] Key to designations for media in Table 3:

I Plain 2 per cent agar plus gas (propylene oxide) sterilized pine needles.
II Potato dextrose agar.
IIA Potato dextrose agar plus 4 drops of lactic acid in each culture plate.
III Plain 2 per cent agar.
IV Cornmeal agar.
IVA Cornmeal agar plus 4 drops of lactic acid in each culture plate.
V Plain 2 per cent agar plus green pine needles sterilized with chloral hydrate.
VI Yeast infusion agar.
VII Pine needle decoction agar.
VIII Fresh green pine needles.
IX Malt agar (2 per cent).

[c] Key to designations for source material in Table 3:

1. Small pieces of diseased needles.
2. Small pieces of hysterothecia.
3. Ascospores from needles soaked in water for 2–3 hours.
4. Ascospores from needles soaked in water for 10 days.
5. Ascospores manually removed from needles soaked in water for 3 days.
6. Same as 5, but soaked only 2–3 hours.
7. Needle containing hysterothecia suspended over medium.
8. Entire contents of hysterothecium removed promptly after hysterothecium opened.
9. Pycnidiospores in water.
10. Pycnidiospores in granular exudate (not dissolved in water).

tubes (Fig. 3, B). Occasionally, these would branch once, but all failed to grow and produce the "abundant white mycelium" described by Weir.

*Histo-pathology.*—The needles and twigs of ponderosa and Jeffrey pines are heavily armored with stone cells and are rather difficult to section without employing a softening technique. In this study, 3 methods of softening the tissue were tried: a) soaking in lactophenol for 2 weeks at room temperature and then for a similar period in a paraffin oven at 53°C. (11); b) treatment in a dental vulcanizer after the method of Jeffrey (8); and c) soaking in hydrofluoric acid (30–40 per cent) for 4 to 8 hours. The latter proved to be the best, because of its simplicity.

Diseased twigs were embedded in parlodion, but this proved too slow and cumbersome to be practical, and so they were split longitudinally and embedded in paraffin, with the standard ethyl alcohol method. Needles were cut in short sections and were likewise embedded in paraffin. After being embedded, both were given additional softening treatment, with Gifford's solution No. 2 (6).

Differential staining of the sections to distinguish the fungus mycelium from the host tissue proved to be rather difficult, since the hyphae stain very lightly. In all, 6 stains were tried: a) Fleming's triple stain (2); b) safranin-light green (13); c) Cartwright's stain (1); d) Foster's iron chloride-tannic acid (9), modified by counterstaining with light green in 95 per cent alcohol; e) phloxine (1 per cent in tap water) with a light green counterstain (9); and f) Delafield's haematoxylin with a safranin counterstain (9). The last one proved to be the most satisfactory for differentiating the fungus hyphae from the needle tissue, whereas the phloxine-light green combination was very good for distinguishing diseased from normal plant tissue.

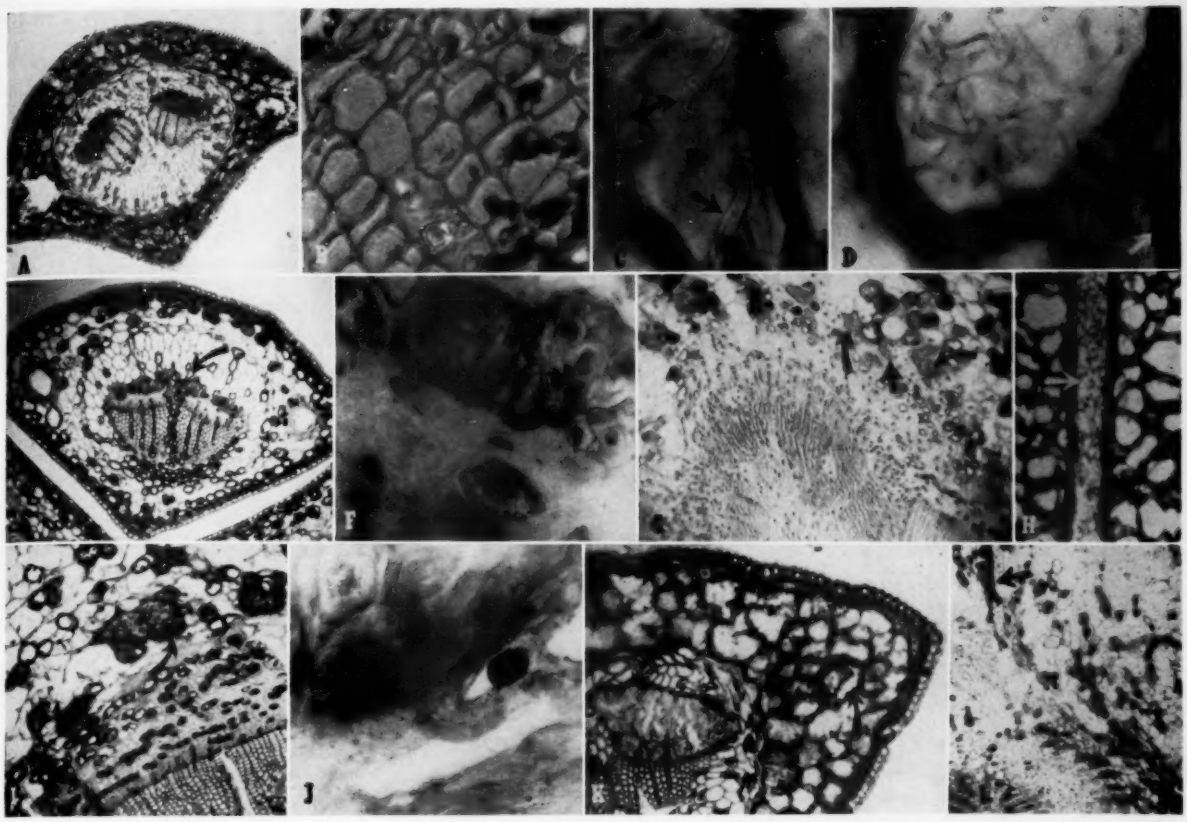

FIG. 4. Photomicrographs of *Elytroderma deformans* and its effects on needles and twigs of *Pinus ponderosa*. Unless stated otherwise, sections are transverse. A) Normal needle (35×). The resin canal on the left was torn in sectioning. B) Normal needle phloem (1000×). C) Parenchyma cell from the mesophyll region, showing fungus hyphae (550×). D) Portion of a mycelium-filled resin canal in needle (500×). E) Needle section cut a short distance above the apex of the short shoot (50×). Note that about ⅓ of the phloem is degenerating. F) Enlarged portion of the phloem indicated by the arrow in Fig. E (400×). G) Section through twig and short shoot at point of juncture, showing groups of degenerating phloem cells at periphery of phloem (50×). H. Tangential section through a needle resin canal, showing fungus hyphae filling the lacuna (150×). I) Portion of parenchyma and xylem from twig, showing groups of degenerating phloem cells in parenchyma adjacent to phloem zone (50×). J) Enlarged portion of degenerating phloem cells shown at tip of arrow in Fig. I (500×). K) Portion of a diseased needle, showing degenerating phloem at the top of the vascular bundle and a mycelium-filled resin canal at the right (100×). L) Tangential section through twig, showing groups of degenerating phloem cells (see arrows) extending from the point of departure of the short shoot (lower left) into the twig (35×).

Invasion of the host by the mycelium of *E. deformans* is apparently limited in extent, being confined to the subepidermal and mesophyll regions in the midsection of the needles. Here the hyphae are concentrated in numbers in only 2 locations: a) directly beneath the fruiting bodies, forming a shallow layer of plectenchyma; and b) within the lacunae of the resin canals (Fig. 4, D and H), particularly those near the outer (curved) surface of the needle. The intracellular mycelium is sparsely scattered within the parenchyma cells (Fig. 4, C) throughout the mesophyll region and it penetrates the cell walls directly in passing from cell to cell. No hyphae have ever been observed in the vascular cylinder or within the endodermis, nor has any been found in the tissues of the stem or twigs. The mycelium appears to extend longitudinally in the mesophyll for only 1 or 2 mm. in each direction beyond the area on which the fruiting bodies are produced, but damage in the form of degenerative phloem tissue extends throughout the entire length of the needle and for an undetermined distance down the twig (Fig. 4, E-G, I, J, and L).

The most striking effect of the disease noted in a transverse section of an affected needle is the degeneration of the phloem tissue (Fig. 4, K). This was illustrated by Weir (15, Fig. 3, p. 280) in the report of his work, but he made no mention of it in the text. Ponderosa and Jeffrey pines, like all hard pines, have 2 collateral vascular bundles, surrounded by transfusion tissue, in the central portion of each needle (Fig. 4, A). Under normal conditions, the cells of the xylem and the phloem tissues composing each bundle remain in more or less discrete radial rows (Fig. 4,B). When the needles are attacked by *E. deformans*, however, the cells of the phloem tissue round out, take a distinctive form and become irreg-

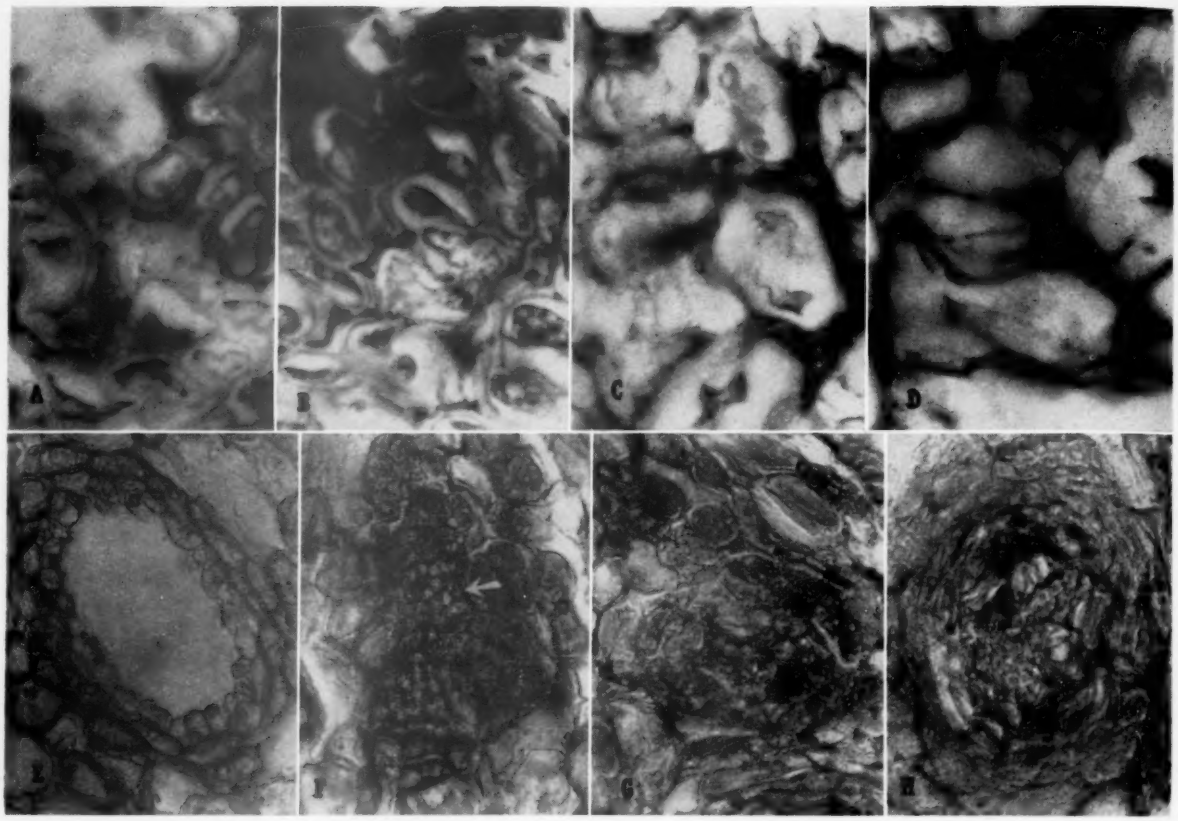

FIG. 5. The effects of *Elytroderma deformans* on *Pinus ponderosa*. A-D) Photomicrographs of stages in the degeneration of needle phloem cells (1000×). A) Walls of affected cells beginning to swell. Note the striate appearance. B) Expansion of the cell contents. Note the deep staining of the cell contents but lack of staining of cell wall. C) Several stages in the degenerative reaction. Note the "plasmolysis" of some cells. D) Dissolution of the middle lamellae and deep staining of the cells walls. Note the indefinite shape of some cells. E) Normal resin canal in the twig bark (150×). F-H) Stages in the early development of traumatic resin cysts (see Fig. 1, B and E) in the bark (150×). F) Degenerating phloem cells with the beginning of cell division at left. G) Later stage in resin cyst formatioin around degenerating phloem cells. H) Fully formed epithelium with a lining of secreting cells, which have already begun to secrete resin into the cyst.

ular in arrangement (Figs. 4, E-G, I-L, and 5, A-D). The sieve cells in the region of heaviest hyphal concentration—i.e., in the vicinity of hysterothecia—further degenerate until the entire phloem bundle in that portion of the needle is a shapeless mass.

The amount of damage to the phloem tissue decreases at progressively greater distances from the infected region. The vascular bundles coalesce below the point of juncture of the needles and the short shoot. At this point, damaged sieve cells form a nearly complete ring around the vascular bundle. These cells can be followed down the length of the short shoot and into the twig (Fig. 4, L).

In affected twigs, groups of degenerated phloem cells (Fig. 4, I and J) can be found in "interwhorls" (stem between 2 successive whorls of laterals) of all ages except the very youngest. No trace of them was found in current year's buds. This perhaps is due to the small amount of mature phloem in the bud, but a more reasonable explanation would seem to be that the movement of substances in the phloem is primarily in a downward direction.

The steps in the degeneration of the phloem tissue are gradual and ill-defined, but may be described as follows: The walls of 1 or more sieve cells along the outer edge of the phloem bundle begin to swell and, under sufficient magnification, have a striate appearance (Fig. 5, A). The content of the cell then expands and is deeply stained by either phloxine or picroanaline-blue, while the walls remain almost colorless (Fig. 5, B). Soon, other cells respond in the same way, the result being a greatly enlarged area of normal and degenerating sieve cells (Fig. 5, C). The middle lamellae begin to dissolve and the walls of affected sieve cells acquire the same affinity for dyes as the protoplasm. The transfusion tissue is crushed and affected sieve cells are pushed apart by the swelling of additional phloem elements. These eventually collapse, forming an indefinite mass of cell walls and secretory material (Fig. 5, D).

Microscopic examination of transverse and tangential-longitudinal sections cut through the brown resin

cysts in the bark, described under symptoms (Fig. 1, B and E), showed the areas to be filled with resin, the composition of which was not determined. The constancy of their occurrence on diseased twigs indicated that they were associated with attacks of elytroderma, and detailed study of the early stages in their formation showed them to be pathological resin cysts (Fig. 5, F-H). The degenerating phloem cells appear to stimulate renewed multiplication in the surrounding parenchyma cells. This multiplication begins with the unequal tangential division of a parenchyma cell adjacent to a group of degenerated phloem cells (Fig. 5, F). Nearby parenchyma cells soon divide in the same manner (Fig. 5, G) until an epithelium, 3 or more cells wide and lined with secreting cells, entirely surrounds the diseased cells (Fig. 5, H), in contrast to a normal resin canal (Fig. 5, E), which consists of a lacuna surrounded by a unicellular row of secreting cells encircled by a double row of epitheclial cells. The secretory cells of the pathological resin cyst, by this time, have started secreting resin into the lacuna of the canal. As the canal becomes filled with resin, the parenchyma cells comprising the epithelium are stretched and pushed out of shape until they are elongated and the lateral walls become convex from the volume of material within. The diseased phloem cells are gradually obliterated by the influx of the resin. The resulting resin-filled cysts are the brown areas in the bark shown in Fig. 1, B and E. Apparently, not all groups of degenerated phloem cells are thus limited. In all material examined, groups of such abnormal cells were found, around which no evidence of pathological resin cyst formation could be discerned.

Discussion.—One of the points concerning the Elytroderma disease about which pathologists and foresters have felt uncertainty is the time of year at which infection of pine needles normally takes place. The fact that the last-formed needles ordinarily turn red early in the spring of the following season suggests that infection probably occurs sometime between the time of opening of the pine buds in the spring and the time of stopping of activity for most fungi by low temperatures in late fall or early winter. Weir's statements, already cited, indicated that infection might occur at almost any season of the year at which moisture and temperature conditions were favorable for the fungus. He noted that temperature was a very important factor in spore discharge, since no spores were liberated at 40°F., but they were released in quantity when the temperature was raised to 80°F.

The inhibiting effect of low temperature on spore discharge is confirmed by the results obtained from the trap slides exposed during the fall and winter of 1950–51 on the Ochoco National Forest in Oregon. The number of spores caught gradually decreased as the weather became colder, even though there was plenty of moisture in the form of rain, dew, and snow. There was no discharge between January 25 and March 20 (Fig. 2), and probably none during any of January, since the set of traps for this period was exposed from December 18 to January 24 and it is probable that all spores caught for the period were discharged late in December. Discharge was resumed at a much lower level between March 20 and May 7 under the influence of the warmer weather prevailing then.

There was an even longer winter break in the occurrence of germinated ascospores on the exposed trap slides, as mentioned earlier in connection with Fig. 2. Thus, even if some discharge of spores were to occur during early winter, infection of needles would be unlikely to take place then, under climatic conditions such as those characteristic for the Ochoco National Forest and other areas where the disease has proved to be severe, because temperatures would be too low to favor germination and establishment of the pathogen.

Infection during the spring months is possible, but the supply of ascospores at that time of year appears to be limited. Since the previous years' needles are involved and begin to fade as early as January or February, the time of infection must be several months earlier. If it occurred during July after the needles emerge from their sheaths, the needles might develop to normal size before the degenerative effects of the fungus on the phloem tissue, which have been demonstrated, set in. However, during this period favorable moisture conditions occur only occasionally and for brief intervals, and infection under these conditions appears doubtful. There is not yet enough evidence to state with certainty that the pycnidiospores do or do not play a role in the dissemination of the disease, and further work is planned to clarify this point.

Failure to obtain the fungus in pure culture, or even to get a reasonable number of ascospores to germinate, is difficult to understand. Weir (15) reported neither the culture medium nor the incubation temperature that he used, so it was impossible to duplicate his work.

Weir's spore germination trials seem questionable for 2 reasons: a) the 35°C. temperature at which the spores were germinated is unreasonably high; and b) no evidence is presented which would indicate that the ascospores he used were ungerminated before his trials began.

The high temperature is particularly open to question. Wolf and Wolf (16) list 33 species of fungi (Table 8, page 99) which they say are "representative", and give the cardinal temperatures for each. Their figures clearly show that the optimum temperature for the development of most fungi is below 32°C. The high temperature also is out of keeping with the altitude, climatic factors, and development of the disease under natural conditions. Most commercial stands of ponderosa and Jeffrey pines in northern California and Oregon grow at altitudes between 4,000 and 6,500 ft., and the air temperatures rarely reach 32°C., even on the hottest days. Temperatures such as Weir used, which perhaps might be reached by needles exposed to the direct rays of the sun, are

not ordinarily associated with moisture sufficient to permit germination.

Additional evidence against the requirement of high temperature is the pattern of the disease in the forest. It is much more prevalent at the higher altitudes than at lower levels. Wagener et al (14) report that "Attacks have usually been heaviest toward the upper altitudinal limits. . . . Most stands at low elevations have remained almost entirely free of the disease. Within affected drainages, damage has been most pronounced in ravine bottoms and along the lower slopes and least on the ridges in most areas."

Recently Mielke[7] repeatedly obtained very good spore germination in 24 hours at temperatures between 10° and 20°C. The temperatures tested varied from 4° to 30°C. Below 10° he found that germination was good but germ tube lengths approximated only ½ the length of the spores. A few branched tubes were observed. Between 10° and 15° the germ tube length was much the same as spore length and branching was common. Germination was only fair at 20°. At 25° very poor germination occurred, the germ tubes were variable in length, they had a distinct tendency to round off at the tips, producing structures similar to those shown in Fig. 3, B, and branching was uncommon. At 30° no germination took place, plasmolysis was severe, and the spores were apparently killed. Thus, the optimum temperature for germination of this spore form seems to be about 15°C., not 35° as reported by Weir.

Darker (3) found that in some cases ascospores of *Hypoderma pini* (Dearness) Darker germinated within the ascus. [See Darker (3, Plate IX, Fig. 6)]. There is ample evidence in my material to show that the ascospores of *E. deformans* can, and often do, germinate within the ascus and also within the hysterothecium (Table 1 and Fig. 3, C-E). In view of my failure to duplicate Weir's work, it seems possible that he may have collected similar material and thought that the germination occurred after his trials began. However, this still does not explain the fungus growth attributed by him to *E. deformans*.

Darker (3) also observed that "Species which are known to be most parasitic in their action . . . do not germinate freely in distilled water." He reported culture work and spore germination trials for various species of *Bifusella*, *Hypoderma*, *Hypodermella*, and *Lophodermium*, and illustrated germinating ascospores for all species in which this phenomenon was observed, but he showed none for *E. deformans*.

Weir (15) states that the growth of the mycelium in the shoot is the cause of spherical-shaped witch's brooms. He also says: "In a far greater measure than in any other member of the order, the mycelium of *H. deformans* penetrates the leaf sheath and eventually perennates in the tissues of the shoot, causing a marked enlargement of the parts infected," but he gives no evidence to support these statements. My investigations have failed to show mycelium of the causal fungus in the shoot. There is little doubt that the witch's brooms are a result of continued infection by this fungus, for such brooms produce needles bearing hysterothecia year after year, even when no signs of the disease appear on other trees. This may be due to 1) the large numbers of shed diseased needles, bearing mature hysterothecia, that are caught and held by the brooms, providing an abundance of inoculum; and 2) the dense condition of these abnormalities which retards drying after rain and dew, thus creating conditions favorable for infection even during years when climatic factors are generally unfavorable for infection of needles other than those associated with a witch's broom.

Jump (10) found that he could satisfactorily explain abnormal forking in red pine (*Pinus resinosa* Ait.) only on the basis of a phytohormone produced by *Dematium pullulans* de Bary living in the buds and branches. Similarly, Mitchell and Angel (12) found that *Fusarium moniliforme* Sheldon in liquid culture produces a substance that markedly stimulates elongation of bean plants. Certain preparations of this material caused a marked twisting and later gall formation on treated plants. Until Elytroderma can be successfully grown in culture, it is impossible to demonstrate that it produces such substances. However, the presence of toxic material is suggested by the pattern of phloem tissue degeneration that has been shown to take place in affected needles and, to some extent, in the twigs of affected pines.

Esau (5), in connection with the degeneration of phloem caused by plant viruses, found that the breakdown of the phloem as a conducting tissue followed more than 1 distinct pattern. She grouped these patterns into 3 tentative categories, based upon: 1) the point of origin of the degeneration; 2) the presence or absence of abnormal growth; and 3) the chronological sequence of the occurrence where both conditions were involved. Her "Group A" includes those in which "degeneration begins with a necrosis of the sieve tubes themselves and is or is not followed by pronounced growth disturbances." This agrees very closely with the sequence of phloem degeneration associated with attacks of *E. deformans* and suggests that her groupings may have an even wider application than she indicates.

From a forestry standpoint, the importance of the Elytroderma disease arises not only from the damage resulting from the repeated heavy infection of trees, but also from the disruption of cutting budgets where salvage operations become necessary, and from the dislocation of timber management plans. Very often, the trees most severely affected are those in the immature class with small diameters and volumes, the type of trees ordinarily left for a future cut, whereas those least affected are in the mature or overmature classes, which are those usually cut in normal timber-harvesting practice. Thus, if anything of merchantable size is to be left as a seed source for the affected stands

[7] See footnote 6, page 562.

or for a future cut, it must be chiefly in the mature and overmature age classes in which growth is slowing down and the risk of bark beetle attack is increasing.

Individual ponderosa pines practically free from the disease are found in heavily affected areas and thus appear to be resistant to it. If such resistance proves to be actual on further test and to be genetically linked, it should be possible to develop races of this pine with highly resistant progeny that could be used in places where the disease is severe. However, stock of this sort could be developed only through a long-time breeding program and offers no present solution for foresters confronted with the results of the recent and continuing epidemic.

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# APHID TRANSMISSION OF THE WISCONSIN PEA STREAK VIRUS[1]

C. B. Skotland and D. J. Hagedorn

SUMMARY

The pea aphid (*Macrosiphum pisi* Ketb.) was found to be an inefficient vector of the Wisconsin pea struck virus, giving low percentages of transmission. Data obtained suggest that it is a non-persistent virus—acquisition threshold period was occassionally 15 seconds; vector efficiency was increased by starving; and the virus did not persist in the pea aphid. The following manipulations failed to increase vector efficiency: acquisition feeding period increase from 1 to 24 hours; use of aphids in different stages of development. Increase in transmission resulted from acquisition feeding aphids on peas exhibiting late disease symptoms compared to milder symptoms. Aphids transmitted the virus from pea to red clover and broad bean and from red clover and yellow sweet clover to pea.

Critical investigations of insect-virus relationships have been made by Osborn for 2 pea viruses—enation pea mosaic virus (4) and the virus he designated as pea virus 2 (5). He reported that the pea aphid (*Macrosiphum pisi* Ketb.) remained viruliferous with the former virus for as long as 29 days but that pea virus 2 was retained by the pea aphid for only 1 hour.

No detailed studies of this kind have been made with the American pea streak virus described by Zaumeyer (7), the New Zealand pea streak virus reported by Chamberlain (1), or the Wisconsin pea streak virus described by Hagedorn and Walker (2). These 3 viruses appear to be unrelated. Results of extensive experiments on insect-vector relationships of the Wisconsin pea streak virus are presented in this paper.

Materials and methods.—This investigation was conducted in a greenhouse at an air temperature of

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approximately 20°C. A 16-hour day was maintained. Daylight was supplemented and extended by 40-watt daylight fluorescent bulbs. Pea plants (*Pisum sativum* L.), variety Perfected Wales, medium red clover (*Trifolium pratense* L.), and yellow sweet clover (*Melilotus officinalis* L.) were employed to maintain stock cultures of the virus and also in the transmission experiments. In the earlier part of the study, the plants were grown in composted soil in 6-in. earthenware pots, and in the later work the peas were grown in sterilized quartz sand. The peas growing in sand were watered with Hoagland's solution (3).

The Wisconsin pea streak virus used in these studies was obtained from desiccated pea leaves containing the original virus described by Hagedorn and Walker (2). Transfers to stock culture plants were made by mechanical inoculation.

Nonviruliferous pea aphids were maintained on healthy peas in aphid-tight wire mesh cages. Several different colonies were used.

The aphids were removed from healthy peas by brushing into a suitable container. In mass transfers, the aphids were enclosed on a group of plants by means of a 30-mesh brass wire cloth cage, approximately 5 in. in diameter, which had a removable cheesecloth top fastened by means of a metal band. When 1 plant was to be infested, the aphids were confined by glass lamp chimneys, the tops of which were covered by cheesecloth. In single aphid experiments, the individual plants were enclosed by glass tubes 1½ in. in diameter. Transfer of viruliferous aphids was usually accomplished by means of a moistened "camels hair" brush.

After the inoculation feeding period the aphids were killed with an insecticidal spray.

EXPERIMENTAL RESULTS. — *Acquisition threshold period.*—Mature nonviruliferous apterous pea aphids, starved for 1–8 hours, were placed on pea plants infected with the Wisconsin pea streak virus. The timed acquisition feeding period (6) began as soon as the aphid placed its proboscis in a permanent feeding position. The viruliferous aphids were then given a 24-hour inoculation feeding period on healthy host plants, 1 aphid per plant. Twenty or more trials were made for each acquisition feeding period.

Progressively higher percentages of transmission were observed as the acquisition feeding period was lengthened. Aphids given an acquisition feeding period of 15 seconds transferred the virus to only 0.32 per cent of 308 plants tested. When aphids given acquisition feeding periods of 1 and 5 minutes were transferred to 405 and 304 plants, the respective percentages of virus transmission were 1.23 and 1.31. Three per cent of 100 plants and 3.27 per cent of 458 plants became diseased when aphids given respective acquisition feeding periods of 1 hour and 24 hours were transferred thereto.

*Non-persistence of the Wisconsin pea streak virus in the pea aphid.*—Mature pea aphid aptera were given a 24-hour acquisition feeding period and then individually transferred to a series of 4 test plants. The inoculation period was 20 minutes on each of the first 3 test plants and 24 hours on the fourth. In a total of 400 trials, 5 aphids transmitted the virus only to the first plant fed upon, 1 aphid transmitted only to the second and another only to the third. In no trial was the fourth plant infected nor did more than 1 plant in a series become diseased. These data suggest that this virus is non-persistent in the pea aphid.

*Other hosts.*—Various biennial legumes, i.e., sweet clover and red clover (2) are possible overwintering hosts of the Wisconsin pea streak virus. In testing transmission to and from these and other suscepts, aphids were given a 24-hour acquisition feeding period and a 24-hour inoculation feeding period. From 15–20 aphids were placed on each of 15–69 plants except for broad bean in which case approximately 100 aphids were placed on each plant.

Wisconsin pea streak virus was transmitted by pea aphids from red clover to about 9 per cent of the pea plants tested; from pea to about 6 per cent of the red clover plants studied; from yellow sweet clover to 6 per cent of the pea plants tested, but not from pea to yellow sweet clover; from pea to 40 per cent of the broad bean (*Vicia faba* L. var. *minor*) plants, but not from pea to alfalfa (*Medicago sativa* L.) var. Ranger.

*Factors influencing transmission of the Wisconsin pea streak virus.*—a) *Length of acquisition feeding period.*—Aphids were given acquisition feeding periods of 1, 4, 8, and 24 hours, then placed on 12–16 healthy plants, 10–15 aphids per plant. They were left on each plant for 24 hours in each of 10 trials.

After 1, 4, 8, and 24-hour acquisition feedings, respectively, and a 24-hour inoculation feeding, 11 of 133 (8 per cent), 9 of 131 (7 per cent), 11 of 121 (9 per cent), and 15 of 142 (10 per cent) plants became diseased. These results indicated no significant differences between virus transmissibility of aphids given the acquisition feeding periods indicated.

b) *Starvation of aphids.*—Pea aphids were starved for 1–2 hours and allowed to feed on diseased pea plants for 15 seconds, 1 minute and 5 minutes, then placed, 1 per plant, on healthy plants for a 24-hour inoculation feeding period. Starved aphids transmitted the virus to 1 of 151, 3 of 202, and 4 of 151 plants for the respective acquisition feeding periods mentioned above. For these same periods non-starved aphids transmitted the virus to 0 of 155, 2 of 203, and 0 of 151 plants tested. Thus, an increase in transmission was achieved by vector starvation, but very slight transmission was obtained even after this manipulation.

c) *Severity of disease in source plants.*—Pea aphids appear to feed more readily on plants showing early symptoms of pea streak than on those in later stages of the disease. This suggested the possibility that physical condition of the source plants, resulting from presence of virus and expressed in severity of disease symptoms, might influence ability of the aphid to pick

up and transmit the virus. Source plants were classified as showing early, medium, or late symptoms.

Pea aphids were allowed a 24-hour acquisition feeding period on plants in each of the 3 symptom classes before being transferred, 1 aphid per plant, to healthy peas where they were given a 24-hour inoculation feeding period.

In 10–50 trials, aphids fed on early, medium, and late symptom source plants transmitted to 3 per cent of 218 healthy plants, to slightly less than 3 per cent of 559 plants, and to 8.2 per cent of 122 plants, respectively. This demonstrated plants with late symptoms to be better sources of virus than those with earlier and milder symptoms.

d) *Age of insect.*—After being given a 24-hour acquisition feeding period on the same diseased host plant, aphids in the first, second, and third instars were transferred to one group of plants and fourth instars and adults to another group, 10–15 aphids per plant. The inoculation feeding period was also 24-hours.

Only about 2.5 per cent of the 371 pea plants on which adults and fourth instar nymphs fed became diseased. Slightly less than 2 per cent of the 292 plants on which aphids in the first, second, and third instar fed became infected. Thus, pea aphid maturity did not appear to be an important factor in transmission of the Wisconsin pea streak virus by this insect.

Discussion.—Although the percentages of transmission were too low to permit conclusive interpretation, the sum total of the data obtained indicates that the Wisconsin pea streak virus resembles non-persistent rather than persistent viruses. The virus appears to be characterized by a short acquisition feeding period, non-persistence in its vector, and apparently is transmitted somewhat more readily by starved aphids than by non-starved ones. Previously published data (2) on its physical properties and transmissibility by mechanical methods also support this view.

Very low percentages of transmission of the virus were obtained throughout this investigation. A number of different sources of pea aphids were used in a variety of experiments and manipulations, but by-and-large, efforts to obtain a high level of transmission were unsuccessful. The exception was observed when viruliferous aphids from peas transmitted the virus to 40 per cent of the broad beans tested. This relatively high degree of transmission was probably due to the larger number of aphids placed on each broad bean plant and/or to the natural affinity these insects have for this host. The low transmission to peas in these greenhouse studies is not inconsistent with observations of field spread of the virus.

It is significatnt that the pea aphid was found capable of transmitting the Wisconsin pea streak virus from red clover and yellow sweet clover to pea and from pea to red clover. The fact that this aphid is found on these widespread virus reservoir plants and transmits the virus to and from these clovers may explain the incidence of Wisconsin pea streak.

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# STUDIES ON THE PATHOGENICITY OF SEVEN SPECIES OF PYTHIUM ON ALFALFA, SWEETCLOVER, AND LADINO CLOVER SEEDLINGS[1]

J. E. Halpin, E. W. Hanson, and J. G. Dickson[2]

SUMMARY

The relative pathogenicity of 7 isolates of *Pythium* was determined on alfalfa, sweetclover, and Ladino clover seedlings in sand culture in the greenhouse at 16°, 20°, 24°, and 28°C. Pathogenicity was measured in terms of effects on stand, rapidity of killing, and disease severity on surviving plants.

*P. debaryanum*, *P. irregulare*, *P. splendens*, and *P. ultimum* were most pathogenic and had similar effects on each of the hosts. *P. debaryanum* and *P. ultimum* caused somewhat more seed rotting but all these isolates killed all the alfalfa and sweetclover seedings and nearly all the Ladino clover seedlings prior to emergence. *P. splendens* was slightly less pathogenic on Ladino clover at 16–24°C. but equally pathogenic at 28°C.

*P. paroecandrum* was less pathogenic than any of the isolates listed above but it killed from 56 to 85 per cent of the alfalfa seedlings, from 70 to 92 per cent of the sweetclover seedlings, and from 65 to 79 per cent of the Ladino clover seedlings in 15 days, depending on temperature. *P. arrhenomanes* had little effect on any of these legumes. It did incite a light tan discoloration of the secondary roots but had no effect on stand and little if any effect on vigor. *P. rostratum* was completely nonpathogenic.

The pathogenicity of *Pythium* on small-seeded forage legumes has been established (1, 2, 3, 4, 5, 6, 7) but little is known concerning the relative pathogenicity of its various species on these crops. The writers (4) have studied the pathogenicity of 7 species of the fungus on red clover (*Trifolium pratense* L.). Buchholtz and Meredith (2) isolated *P. debaryanum*, *P. splendens*, *P. pulchrum*, *P. rostratum*, and *P. ultimum* from field-grown, damped-off alfalfa seedlings, found them to be pathogenic, but did not indicate their relative pathogenicity. Buchholtz (1), however, con-

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TABLE 1.—*Effects of 7 species of Pythium on stand and disease development on alfalfa, sweetclover, and Ladino clover seedlings grown in the greenhouse at 4 different temperatures for 15 days*

| Host and species of *Pythium* | Average stand in per cent and disease severity rating at indicated temperatures | | | | |
|---|---|---|---|---|---|
| | 16°C. | 20°C. | 24°C. | 28°C. | Average for 4 temperatures |
| Alfalfa | | | | | |
| None (check) | 90 (0)[a] | 92 (0) | 90 (0) | 92 (0) | 91 (0) |
| *P. rostratum* | 92 (0) | 91 (0) | 90 (0) | 91 (0) | 91 (0) |
| *P. arrhenomanes* | 93 (1−) | 93 (1−) | 92 (1−) | 93 (1−) | 93 (1−) |
| *P. paroecandrum* | 25 (2+) | 40 (2+) | 30 (3+) | 14 (4−) | 27 (3) |
| *P. splendens* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. irregulare* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. ultimum* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. debaryanum* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| Sweetclover | | | | | |
| None (check) | 76 (0) | 74 (0) | 72 (0) | 73 (0) | 74 (0) |
| *P. rostratum* | 76 (0) | 73 (0) | 74 (0) | 72 (0) | 74 (0) |
| *P. arrhenomanes* | 76 (1−) | 72 (1−) | 70 (1−) | 71 (1−) | 72 (1−) |
| *P. paroecandrum* | 17 (2+) | 22 (2+) | 10 (3+) | 6 (4−) | 14 (3) |
| *P. splendens* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. irregulare* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. ultimum* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| *P. debaryanum* | 0 (4) | 0 (4) | 0 (4) | 0 (4) | 0 (4) |
| Ladino clover | | | | | |
| None (check) | 81 (0) | 82 (0) | 82 (0) | 81 (0) | 82 (0) |
| *P. rostratum* | 81 (0) | 79 (0) | 81 (0) | 82 (0) | 81 (0) |
| *P. arrhenomanes* | 81 (1−) | 80 (1−) | 81 (1−) | 79 (1−) | 80 (1−) |
| *P. paroecandrum* | 22 (3−) | 19 (3−) | 17 (3−) | 28 (3−) | 22 (3−) |
| *P. splendens* | 6 (4−) | 4 (4−) | 3 (4−) | T[b] (4−) | 3 (4−) |
| *P. irregulare* | T (4−) | 1 (4−) | 1 (4−) | 1 (4−) | 1 (4−) |
| *P. ultimum* | T (4−) | 1 (4−) | 1 (4−) | 2 (4−) | 1 (4−) |
| *P. debaryanum* | 1 (4−) | 1 (4−) | T (4−) | 1 (4−) | 1 (4−) |

[a] Severity ratings are given in parenthesis and based on emerged seedlings only except where no plants emerged; 0 = no disease and 4 = all plants killed either before or after emergence.

[b] T = less than 0.5.

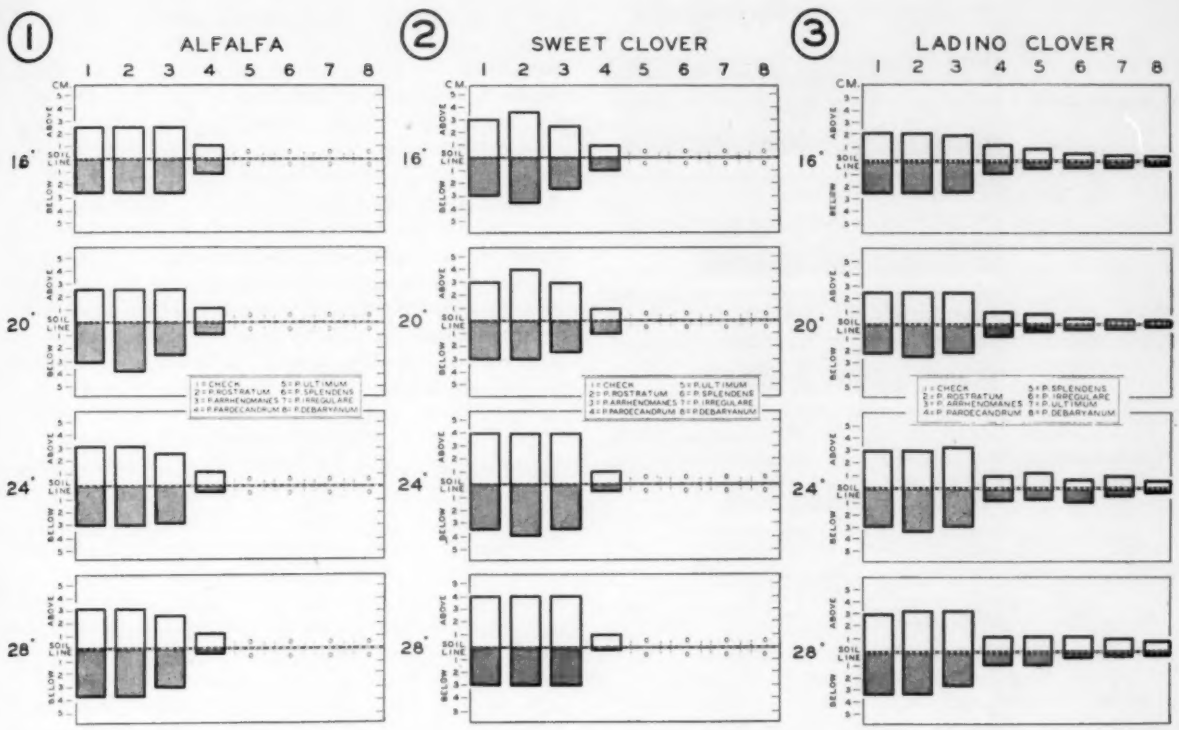


FIG. 1–3. Average length in centimeters of above and below ground parts of legume seedlings still alive 15 days after inoculation with 7 species of *Pythium* at 4 different temperatures.—Fig. 1. Alfalfa.—Fig. 2. Sweetclover.—Fig. 3. Ladino clover.

cluded that *P. debaryanum* was the primary incitant of damping-off of alfalfa in Iowa in 1934, 1936, and 1937. Sprague (6) showed that *P. debaryanum*, *P. ultimum*, *P. monospermum*, and *P. irregulare* could cause 100 per cent killing of alfalfa seedlings in 24 days in greenhouse tests, whereas *P. arrhenomanes* was nonpathogenic on this crop. At least several species of *Pythium* are known to occur in Wisconsin soils and it was the object of this investigation to obtain further information on the relative pathogenicity of some of these species on alfalfa (*Medicago sativa* L.), sweetclover [*Melilotus officinalis* (L.) Desr.] and Ladino clover (*Trifolium repens* L.).

MATERIALS AND METHODS. — The same isolates (Table 1) of *Pythium* used in the earlier studies with red clover (4) were used in this investigation. Their source and relative growth rates at different temperatures were given in the earlier paper. Only 1 isolate of each species was tested. The seed lots used were Naragansett alfalfa produced in California in 1951, Common Yellow sweetclover harvested in Canada in 1951, and Commercial Ladino clover produced in Oregon in 1951. All were free from seed injury and seed-borne fungi. Germination on blotters was similar to that indicated for stand in the checks (Table 1). The glass tumbler technique (4) was used as previously described except that sterile, distilled water was substituted for Hoagland solution in preparing the substratum. One hundred seeds were sown per tumbler. All tests were repeated 3 times with consistent results. Each trial included 4 replicates at each of 4 temperatures, 16°, 20°, 24°, and 28°C., and was of 15 days duration. Data recorded included stand counts, disease severity ratings, observations on symptoms, and measurements of roots and above ground parts of the seedlings.

RESULTS.—The effects of the 7 isolates tested on stand and disease severity are summarized in Table 1. *P. debaryanum*, *P. ultimum*, *P. splendens*, and *P. irregulare* killed all plants of alfalfa and sweetclover prior to emergence in every test. These species were also very pathogenic on Ladino clover, killing nearly all seedlings prior to emergence and most of the survivors soon after emergence. *P. paroecandrum* was less pathogenic than the above but caused average reductions in stand of 81, 73, and 70 per cent for sweetclover, Ladino clover, and alfalfa, respectively. *P. arrhenomanes* had no effect on stand and little if any effect on vigor of any of the crops but did incite a very light tan discoloration of the secondary roots. *P. rostratum* was completely non-pathogenic in all tests.

Fig. 1, 2, and 3 show the effects of the isolates on seedlings still alive 15 days after inoculation. *P. rostratum* caused no reduction in the development of either roots or above ground parts of the seedlings.

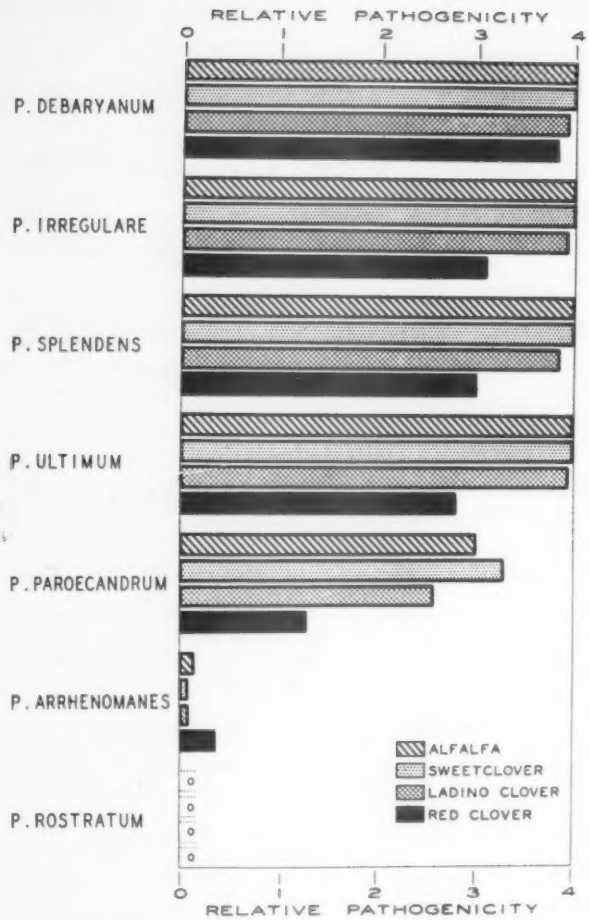


FIG. 4. Graphic summary of the relative pathogenicity of 7 isolates of *Pythium* on alfalfa, sweetclover, Ladino clover, and red clover. Summary based on effects on stand, rapidity of killing, and disease severity on surviving plants.

*P. arrhenomanes* also had little if any effect on the size of plants. *P. paroecandrum* caused severe stunting and almost completely destroyed the roots of alfalfa and sweetclover at the higher temperatures. Ladino clover was similarly affected, but not quite so severely. Since *P. splendens*, *P. irregulare*, *P. ultimum*, and *P. debaryanum* killed all plants of alfalfa and sweetclover prior to emergence there were no survivors to measure. These isolates also killed most Ladino clover seedlings prior to emergence. Surviving plants were severly stunted.

Symptoms were similar to those reported for red clover (4). In general, all these legumes were more susceptible than red clover (Fig. 4). Alfalfa and sweetclover were most susceptible and Ladino clover was intermediate between these and red clover. There was little difference between the amounts of disease on alfalfa and sweetclover. Whereas these comparisons were based on a single seed lot of each host, similar results have also been obtained in other studies with more seed lots. *P. arrhenomanes* was a little more pathogenic on red clover than on the other legumes but was of little consequence on any of these crops.

Temperature had no significant effect on total emergence in the checks. However, seedlings emerged sooner at the higher temperatures and were taller at the end of the tests. Seedlings grown at the lower temperatures had the best roots. There was little apparent effect of temperature on pathogenicity in most cases, probably due to the very high level of disease obtained at all temperatures. Disease was somewhat more severe at the higher temperatures.

More work is needed to determine the range in pathogenicity among different isolates of each of these species. Sprague (6) has presented strong evidence for the existence of distinct races in *P. ultimum*, *P. debaryanum*, and *P. irregulare*. Sprague and Atkinson (7) tested a large number of isolates of *P. arrhenomanes* and found no clearly defined differences in pathogenicity. Further study (6) verified their observations to a great extent, but Sprague (5) did find 1 nontypical form of *P. arrhenomanes* which was more pathogenic on certain grasses than were typical forms of the fungus. Buchholtz and Meredith (2) reported that *P. rostratum* isolated from damped-off alfalfa seedlings was pathogenic. They did not state what hosts were used in determining this pathogenicity but if their isolates incited any disease on alfalfa, sweetclover, Ladino clover, or red clover, they were different from the isolate used by the writers.

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# SOME CONDITIONS INFLUENCING THE RESULTS FROM CORN SEED TREATMENT TESTS[1]

Benjamin Koehler[2]

SUMMARY

One of the important factors in determining damage from seedling diseases in corn was found to be the prevalence of broken places in the pericarp. Pythium could be isolated from the scutellar area of some seeds with broken pericarps in only 3 days after planting in moist field soil at 50°F. Commercially processed seed was found to have pericarp injuries in about 81 per cent of the kernels. A staining technique was necessary to find many of the broken places. In a 3-year field test, commercially processed seed, seed with pericarps partially removed from the crowns in the laboratory, or immature seed showed a significant response to seed treatments with fungicidal protectants. Mature seed with sound pericarps did not respond in either stand or yield. The genetic constitution and age of the seed were other important factors influencing susceptibility to seedling blight and response to seed treatment.

In controlled low temperature tests, germinating conditions could be made sufficiently adverse that seed with sound pericarps suffered considerable loss in stand unless protected by a suitable fungicide. For example, stands of untreated seed with sound pericarps after 20 days in a cold chamber were approximately equivalent to stands obtained with untreated seed with injured pericarps kept in the cold room for a little less than 4 days.

The "cold test" was found valuable for screening new seed treating compounds and determining comparative efficiency of compounds under very adverse germinating conditions, but certain other information could be obtained only by field tests in which plants were grown to maturity.

In cold tests, stand counts alone usually have served as a good measure for determining differences in treatments. This is because differences in stand have been closely correlated with differences in vigor. This did not hold, however, when germination was retarded by low moisture rather than low temperature. In field tests, stand differences due to treatment were usually closely correlated with yields, but there were exceptions when differences were expressed in stand only or in yield only.

Soil sufficiently dry to retard seed germination was found to be conducive to an increase in seedling disease, which was manifested principally by loss in vigor. This was especially true when the pericarp was injured, and the severity of the damage was in proportion to the length of time the seed was in dry soil before adequate water was added. Seed treatment gave partial control.

To determine benefits that might be expected from seed treatment, the use of field tests with commercially processed seed of widely used hybrids and normal practice of time and rate of planting are emphasized.

Many variables may enter into the technique of conducting seed treatment tests with fungicides for the control of seedling diseases. These variables are concerned with the seed condition with respect to pericarp injury, age, maturity, resistance to seedling blight, frost damage, method of drying, and others. Environmental conditions such as the soil fungus flora and temperature and moisture of the soil are important, also. Some of these differences are responsible for great variations in results. The significance of some of the variables is discussed in this paper. Attention is called to the importance of considering these factors in drawing conclusions and also the need of stating the conditions when reporting results. Emphasis is placed on conducting tests that give an accurate indication of the benefit the farmer may expect from the application of a good fungicidal seed treatment. For the sake of brevity not all data in support of statements are presented, and only some of the pertinent literature is cited.

SEED CONDITION.—*Pericarp injuries.*—Broken places in the pericarp allow rapid entrance of soil fungi causing seedling diseases. Therefore, a consideration of the pericarp condition of the seed chosen for seed treatment tests is of great importance in interpreting the results obtained. As early as 1924, Meyers (18) reported that when sound kernels and those with broken seed coats were inoculated with a species of *Penicillium* before planting, the sound kernels showed no effect whereas the injured ones had serious seedling blight damage. In the same year the writer discovered that induced virescence of corn seedlings caused by *Aspergillus flavus* and *A. tamarii* (15) was dependent on pericarp injuries. In 1935, the writer (12) reported that injuries of various kinds at the crown resulted in loss of stand and yield and that the damage could be partially overcome by treating the seed with a protecting fungicide.

More pericarp damage was found in commercial hybrid seed than in well-prepared open-pollinated seed lots (8). This difference was attributed to more extensive use of machinery in harvesting and processing of the seed. In 1948, the writer (9) tabulated various types of pericarp injuries according to their respective importance. Tatum (27) and Tatum and Zuber (28) reported the importance of breaks, even though very slight, in the embryo area of the kernel. Wortman and Rinke (29) found a definite trend toward increased injury as processing progressed,

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TABLE 1.—*Increase in stand and yield of corn resulting from seed treatment as affected by seed condition, and kind and rate of treatment (average time, planting to emergence, 9 days, 2 hybrids, Urbana, Illinois, 1949-1951, incl.)*

| Seed condition code[a] | Seed treatment | Rate | Stand | | Acre yield | |
|---|---|---|---|---|---|---|
| | | | Actual | Increase over untreated | Actual | Increase over untreated |
| | | oz./bu. | per cent | per cent | bushels | bushels |
| A | None | — | 84.1 | — | 87.1 | — |
| (Mature, | Arasan SF[b] | ½ | 91.6 | 7.5 | 94.3 | 7.2 |
| processed) | Arasan SF[b] | 1 | 92.0 | 7.9 | 95.9 | 8.8 |
| | Spergon DDT-SL[c] | ½ | 89.0 | 4.9 | 93.1 | 6.0 |
| | Spergon DDT-SL[c] | 1 | 89.6 | 5.5 | 93.7 | 6.6 |
| | Phygon XL-DDT[d] | ½ | 89.8 | 5.7 | 94.0 | 6.9 |
| | Average increase | | — | 6.3 | — | 7.1 |
| B | None | — | 80.9 | — | 84.7 | — |
| (Mature, | Arasan SF | ½ | 92.2 | 11.3 | 94.8 | 10.1 |
| pericarps | Arasan SF | 1 | 93.8 | 12.9 | 96.3 | 11.6 |
| artificially | Spergon DDT-SL | ½ | 87.7 | 6.8 | 92.3 | 7.6 |
| injured) | Spergon DDT-SL | 1 | 89.7 | 8.8 | 94.1 | 9.4 |
| | Phygon XL-DDT | ½ | 92.0 | 11.1 | 93.2 | 8.5 |
| | Average increase | | — | 10.2 | — | 9.4 |
| C | None | — | 93.6 | — | 96.9 | — |
| (Mature, | Arasan SF | ½ | 94.9 | 1.3 | 97.7 | 0.8 |
| sound | Arasan SF | 1 | 95.1 | 1.5 | 97.6 | 0.7 |
| pericarps) | Spergon DDT-SL | ½ | 93.9 | 0.3 | 96.6 | —0.3 |
| | Spergon DDT-SL | 1 | 94.3 | 0.7 | 98.2 | 1.3 |
| | Phygon XL-DDT | ½ | 94.0 | 0.4 | 96.2 | —0.7 |
| | Average increase | | — | 0.8 | — | 0.4 |
| D | None | — | 81.8 | — | 86.5 | — |
| (Immature, | Arasan SF | ½ | 91.6 | 9.8 | 96.2 | 9.7 |
| sound | Arasan SF | 1 | 91.6 | 9.8 | 95.4 | 8.9 |
| pericarps) | Spergon DDT-SL | ½ | 88.7 | 6.9 | 91.5 | 5.0 |
| | Spergon DDT-SL | 1 | 90.4 | 8.6 | 93.1 | 6.6 |
| | Phygon XL-DDT | ½ | 90.3 | 8.5 | 93.1 | 6.6 |
| | Average increase | | — | 8.7 | — | 7.4 |
| L.S.D., 5 per cent level | | | | 3.2 | | 3.3 |

[a] See Table 2 for seed condition data.
[b] Thiram 75 per cent.
[c] Chloranil 92 per cent plus DDT 3 per cent.
[d] 2,3-dichloro-1,4-naphthoquinone 50 per cent plus DDT 3 per cent.

shelling being the most damaging of the various processing procedures involved.

Corn seed treatment tests with commercially processed seed have been made at the Illinois Station since 1940. Usually several popular hybrids were in the test, each obtained from a different seed producer who had processed the seed except for the fungicidal treatment. This seed, primarily because of pericarp injuries but apparently also due to hot air drying (16, 17), was more subject to seedling blight diseases than hand-picked seed which had been rack dried and carefully handled (Table 1).

When planted in field soil and held at 50°F., *Pythium debaryanun* could be isolated from the embryo region of 3 per cent of crown-damaged kernels in 3 days and, 37 per cent in 4 days, when the seed was untreated. In seed with a sound pericarp, untreated, embryos remained free from any infection until the eighth day, at which time 7 per cent of the kernels revealed *P. debaryanun* in the scutellar area. The plumules had cracked the pericarp on the sixth day.

The observable prevalence of pericarp injuries in processed seed depends on the method of examination. Immersing and stirring the seed sample for 30 seconds in a 0.1 per cent aqueous solution of fast green followed by washing in several changes of water and drying revealed many small cracks that were not seen with a 7 × magnifier (Table 2). Although very small pericarp cracks usually are of some importance, the larger, more easily seen injuries at the same location on the kernel are of greater importance (9).

*Resistance to seedling blight.*—In studies using seed of inbreds, single, double, and 3-way crosses by the writer (unpublished) and others (23, 27) it was found that there were wide differences in resistance to seedling blight. In Illinois tests, differences among results using seed having a sound and injured pericarp within the same inbred were as great as the differences among diverse genetic materials with the same pericarp condition. This emphasizes the importance of knowing the pericarp condition of the seed to be used in a test.

Corn hybrids used widely in the Corn Belt show marked susceptibility to seedling blight when seed with injured pericarps is planted untreated. Whereas it is conceivable that genetic material could be produced that is so highly resistant that seed treatment will become unnecessary, that day is a long way off

TABLE 2.—*Description of seed used in corn seed treatment experiments 1949-1951 incl., average of 2 hybrids*

| Seed condition, code | Maturity | Drying condition | Seed preparation | Pericarp injured kernels | | |
|---|---|---|---|---|---|---|
| | | | | Examined with magnifier but without staining | Stained with fast green | Kernel weights (12% moisture) |
| | | | | per cent | per cent | grams |
| A | Mature | Commercial drier | Machine processed | 42.3[a] | 81.4 | 0.330 |
| B | Mature | Rack dried, room temp. | Handled by hand | 100.0[b] | 100.0 | 0.323 |
| C | Mature | Rack dried, room temp. | Handled by hand | 0 | 6.0 | 0.327 |
| D | Immature | Rack dried, room temp. | Handled by hand | 1.7 | 22.5 | 0.246 |

[a] Injuries resulting from harvesting, shelling, and processing operations.
[b] Pericarp partially shaved off the crowns with a knife.

because the plant breeders, rightly, are devoting attention to obtaining other desired characters including resistance to other important diseases that cannot be controlled, at least to any considerable extent by seed treatment. The very susceptible inbreds, Indiana WF9, Kansas K4, Ohio O7, and others will not be quickly replaced.

*Seed maturity.*—That immature seed is more susceptible than mature seed to seedling blight has been shown by field inoculation experiments (14) and cold tests (26). In the experiment reported here an effort was made to harvest and handle the immature seed in such a way as not to injure the pericarp. Comparisons in field stand and yield were made between immature seed and 1) mature seed with injured pericarps, and 2) mature seed with sound pericarps, to determine if seed treatment could correct for immaturity in field performance.

The immature seed was hand picked from commercial seed producing fields September 5–10 when about 90 per cent of the kernels were denting and the grain moisture was about 42 per cent. The mature ears were picked from the same fields October 10–15. In each case only well-developed ears free from any sign of rot, worm, or bird damage were taken. These were handled carefully to avoid pericarp damage, and were dried at room temperature in racks with air movement provided by a fan. Later, samples of commercially processed seed (without fungicidal treatment) were obtained from the same fields.

The immature seed with a low percentage of pericarp injury gave results similar to the mature seed with a high percentage of injury, with respect to stand, yield, and response to seed treatment (D compared with A and B, Table 1). Arasan, Phygon, and Spergon ranked in the same order of effectiveness on each kind of seed. When part of the pericarp was removed from the crowns of immature seed, blight was more serious and a greater response was noted from the seed treatment. Seed so injured was not tested in field plots but in a cold test only.

Immature seed which had been treated did not perform so well in stand and yield as the mature seed similarly handled to prevent pericarp injuries (D compared with C, Table 1). One point of dissimilarity in seed condition here, in addition to maturity, is a difference in pericarp cracks, even though an effort had been made to prevent injury. The immature seed, when stained, revealed 22.5 per cent injury (Table 2). Nearly all this injury was in the area near the tip end of the kernel. Considerable wrinkling and folding of the pericarp takes place in immature seed during the drying process, and these cracks seem to have developed spontaneously. Injuries near the tip end of the kernel where the radicle emerges are, however, not nearly so important, pathologically, as injuries in some other areas (9, 28).

*Age of seed.*—Other things being equal, the older the seed when planted the poorer the stand (19) and the weaker the plants. Also, seed treatment becomes more effective as the age of the seed increases. In a cold test at 50°F. for 12 days, using untreated seed of hybrid U.S. 13, with sound pericarps aged ½, 1½, and 2½ years, the stands were respectively 83, 55, and 40 per cent. Treated with Arasan the stands were 100, 99, and 98 per cent. When seed was used with the pericarp partially removed from the crown in a 7-day cold test, the stands were respectively 18, 8, and 3 per cent when untreated, 95, 91, and 83 per cent when treated. Thus, when comparing the resistance to seedling blight, or response to seed treatment of various inbreds or hybrids, an important error is introduced if the seed is not of the same age.

*Seed infection.*—Internal infection in processed but untreated seed obtained from seed producers was determined in 50 samples used for seed treatment tests during the years 1940 to 1953 inclusive. Two hundred seeds from each sample were surface sterilized and plated on potato-dextrose agar, 5 kernels per Petri plate. Immersion for 10 minutes with agitation in a solution containing 1 part of a commercial disinfectant containing 3.5 per cent sodium hypochlorite to 9 parts of water and was used for surface sterilization. The percentage of *Diplodia zeae* and *Gibberella zeae* obtained in this way was observed to be very similar to that obtained by methods in which surface sterilization is not used. On the other hand much of the *Fusarium moniliforme, Cephalosporium acremonium,* and *Penicillium* infestation is more superficial, and a more sensitive method of testing the prevalence of infested kernels no doubt would have shown the percentages to be considerably higher.

The following figures are percentages of infection found for the fungi named. The first figure is the maximum infection found in any seed lot for that fungus, the second figure is the average for the 50 seed lots: *Diplodia zeae* (Schw.) Lev., 6.0–.78, *Gibberella zeae* (Schw.) Petch, 3.5–.14, *Fusarium moniliforme* Sheldon, 23.5–6.4, *Cephalosporium acremonium* Cda., 26.5–4.8, *Nigrospora oryzae* (Berk. & Br.) Petch, 13.0–1.7, and *Penicillium* sp., 21.0–.94. Frequently in seed lots the percentage of other miscellaneous fungi, including unidentified ones, outnumbered any one of the above-named fungi, and in a few cases these outnumbered all of those named above. *D. zeae* and *G. zeae* when they occur in viable untreated seed are known to be capable of causing serious seedling blight (5). However, some of the kernels infected with these fungi may be dead, thus limiting the usefulness of seed treatment to that extent. *N. oryzae* may cause seed rot in cold soil. However, the percentage of infection with these fungi, with a few exceptions, was so low in the commercial seed lots that increases in stand and yield due to their control hardly would have been measureable .

Dosage.—Usually it is desired that a known dosage of the fungicide be used in the experiments. To make sure that the seed contained this dosage and that it had not been rubbed off in the hand-planting process, the writer used a 2.5 per cent methyl cellulose solution as the liquid portion of the slurry preparation. This attaches the fungicide so it will not readily rub off and the methyl cellulose itself has no biological effect.

Experiments to determine the best dosage of fungicidal protectant for commercial use, when made by means of severe cold tests, may lead to exaggerated values and should be checked by field tests. Otherwise, the amount of protectant indicated may actually be beyond the point of diminishing returns. Allowance for the most adverse weather conditions that can be expected after corn planting time once in 10 years should be made, but it does not seem wise to go much beyond that margin. There probably is a regional difference with respect to the best dosage, the colder the soil at planting time, the greater the dosage required.

Controlled low temperature tests.—A walk-in refrigerator kept at 50°F. and dark silt loam prairie soil obtained from a cultivated field were used for the controlled low temperature tests, henceforth referred to as "cold tests." Wooden trays with sides 3½ in. high by ¾ in. thick were filled with soil 1¾ in. deep. The seed was then planted and covered with 1¼ in. of soil. Before the trays were filled, the moisture content of the soil was adjusted to the damp margin of the favorable working range for the soil and no additional moisture was added. Soaking the planted trays with water would have hastened seedling blight a little, but the end result would have been the same, as the amount of blight obtained was under complete control by the length of exposure to cold temperatures. The trays were stacked solid in the cold chamber to prevent evaporation. There was no emergence of the plants at that temperature in 20 days. After removal from the cold chamber the trays were placed in a greenhouse with a temperature maintained at 70–75°F., watered as needed, and data taken when the strongest plants had the third leaf well developed but before the fourth leaf showed (Fig. 1). Hoppe (7) reported a simplified cold-testing technique which apparently is well suited for certain purposes.

The length of time desired for the cold test depends on various conditions (25). It is well to choose an interval in which the emergence of the most susceptible material is not completely inhibited. Generally, inbreds or crosses with an inbred as the female parent require less time than hybrids with a cross as the female parent. In some soils blight occurs more rapidly than in other soils. Due to these and other variables,

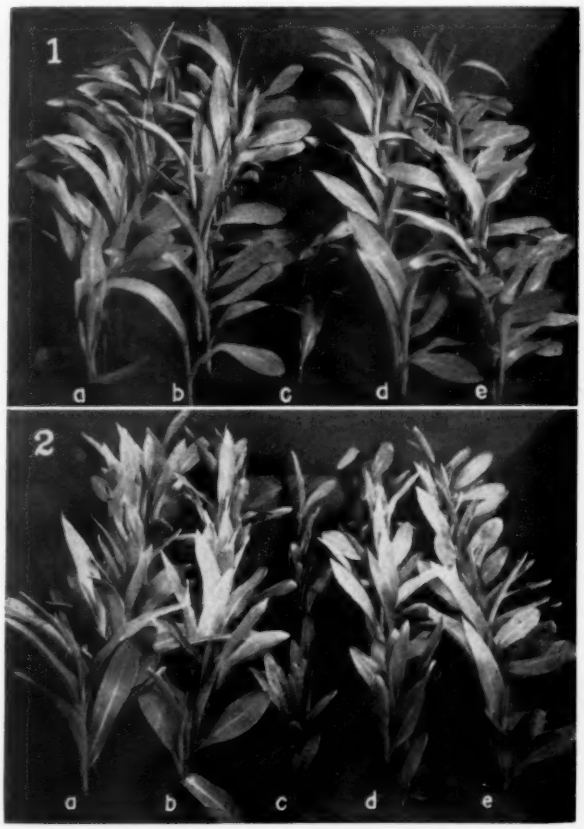


Fig. 1–2. Fig. 1. Growth retarded by cold temperature for 10 days at 50°F. Thereafter growth was accelerated by placing the tray in a greenhouse at 70-75°F. Fig. 2. Growth retarded by having the soil dry for 10 days after which adequate moisture was supplied. The temperature was warm (70–75°) throughout. Both figures: rows a and b grown from seed with a sound pericarp; c, d, and e, similar seed but pericarp partially removed from the crown; b and c, no seed treatment; a and d, Arasan ½ oz. per bushel; and e, Arasan 2 oz. per bushel. Note loss of vigor but no loss in stand in Fig. 2, c. The results in Fig. 1 were caused largely by Pythium species, while those in Fig. 2 were caused by other, unidentified, organisms.

no standard length of time can be set, but has to be determined by trial. In some tests at Illinois it was determined that with crown-injured seed, subjection for a little less than 4 days at 50°F. gave stands similar to those from seed with a sound pericarp after 20 days in the cold chamber when untreated seed was used.

In cold tests of seed lots that differ in amount of pericarp damage or in resistance to seedling blight, there usually is a striking impairment in stand as well as in vigor. In Illinois tests this result has been found to be caused primarily by infection with *Pythium* species (Fig. 1). These differences in vigor have been measured both in terms of number of leaves produced and green weight of plants. When making comparisons between plants from untreated and variously treated seed from a uniform number of seeds planted, there have been close correlations between stand, number of leaves produced, and green weight of plants per row. Thus a stand count alone reflected differences quite well, although the differences with other measurements were more pronounced. The other measurements showed wider differences because the reductions in number of leaves and green weight occurred on the per plant basis as well as per row.

Differences in germination and vigor of seed lots that appear *after* subjection to 50°F. in cold, wet soil, but not at optimum temperature for rapid germination are due to *disease damage*. The differences are not due to "cold resistance", "cold hardiness", and similar terms sometimes found in the literature.

Low soil moisture tests.—Experiments were conducted in which germination was retarded by planting seed of several hybrids in soil with a moisture content slightly higher than the wilting point. The object was to observe the effect on seedling disease development and control by seed treatment. The soil was of the same type as that mentioned above. It had a moisture equivalent of about 28 per cent and the wilting point was calculated to be about 12 per cent, on the oven-dry basis. The tests were conducted in a greenhouse in which the temperature was maintained at 70–75°F. After planting, the trays were stacked to prevent water loss. After 5, 10, and 15 days, trays were spread out and watered until the additional moisture had penetrated to the bottom of the tray. A soil moisture content of 13 per cent was very conducive to seedling blight development. The effect of disease was a little less noticeable at 10.3 and 15.5 than at 13 per cent when kept at those moistures for the same length of time. At 13 per cent moisture the

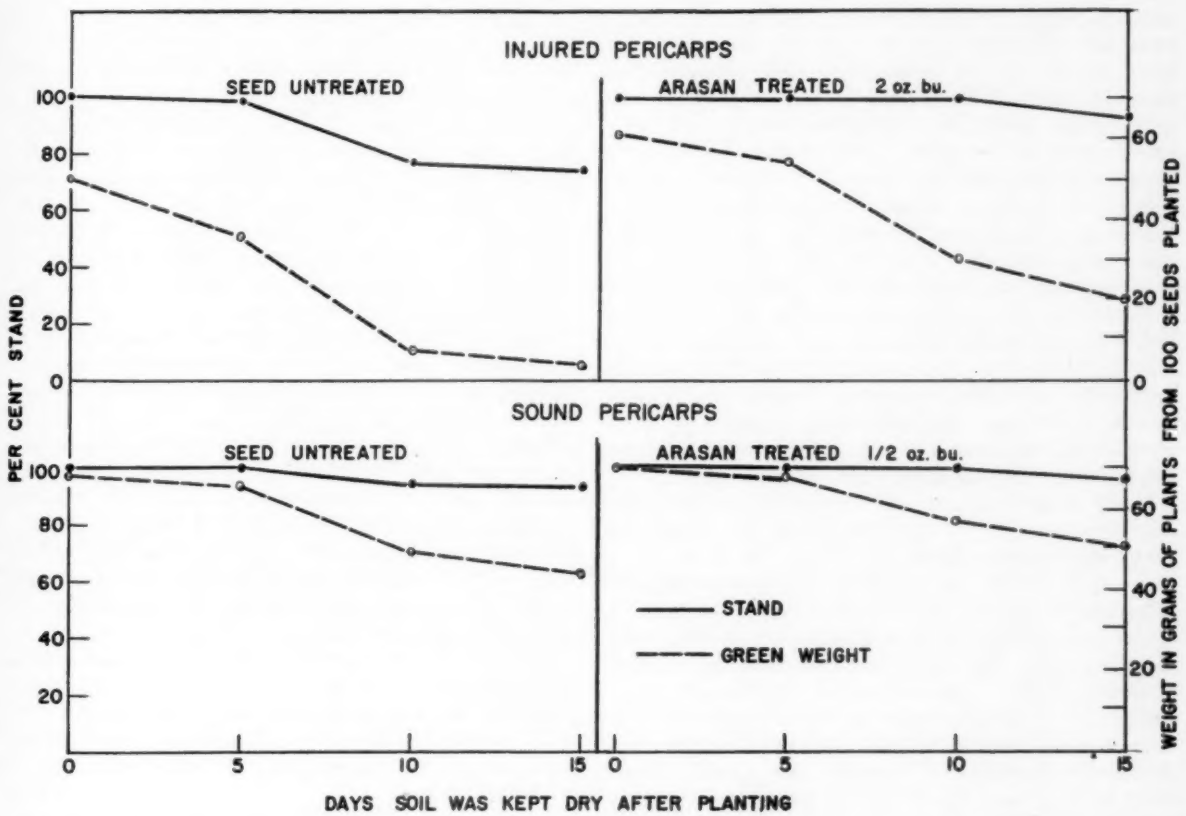


Fig. 3. Differences in stand and green weight associated with planting treated and untreated seed corn with sound and injured pericarps, respectively, in dry soil which was watered to good moisture after various periods of time. Stands and green weights were determined 14 days after watering.

TABLE 3.—*Stands of corn as affected by kind and rate of seed treatment in field tests and in controlled low temperature tests conducted for 2 years. Field tests planted May 11, 1950, and May 5, 1951, (average time from planting to emergence, 9 days), Urbana, Illinois*

| Seed treatment | Rate | Sound pericarp | | Pericarp removed from crown | |
|---|---|---|---|---|---|
| | | Field plots | Cold test 20 days | Field plots | Cold test 10 days |
| | oz./bu. | per cent | per cent | per cent | per cent |
| None | — | 94.4 | 70.4 | 78.9 | 29.4 |
| Arasan SF[a] | ½ | 95.2 | 94.6 | 91.2 | 95.8 |
| Arasan SF[a] | 1 | 95.9 | 95.0 | 93.8 | 98.3 |
| Arasan SF[a] | 2 | ..... | 95.8 | ..... | 98.7 |
| Spergon DDT-SL[b] | ½ | 94.9 | 88.8 | 85.5 | 70.4 |
| Spergon DDT-SL[b] | 1 | 95.2 | 96.7 | 88.4 | 85.8 |
| Spergon DDT-SL[b] | 2 | ..... | 97.5 | ..... | 91.6 |
| Carbide & Carbon #5400[c] | ½ | 92.9 | 95.8 | 88.7 | 97.1 |
| Carbide & Carbon #5400[c] | 1 | 95.9 | 96.2 | 91.7 | 99.1 |
| Carbide & Carbon #5400[c] | 2 | ..... | 99.2 | ..... | 3.8 |
| L.S.D., 5% level | | Not sig. | 3.8 | 3.9 | |

[a] Thiram 75 per cent.
[b] Chloranil 92 per cent plus DDT 3 per cent.
[c] Alpha, alpha-trithiobis 75 per cent (experimental).

radicles had emerged from the seeds after 5 days and the plumules started breaking through the pericarp on the seventh day.

The effect of seedling disease in dry soil was much more evident as reduced seedling vigor than as loss of stand (Fig. 2, 3).

Seeds with injured pericarps were the ones primarily affected. When soil was dry for 10 or 15 days, treatment of seed with injured pericarps increased the green weight per row of plants fourfold. Even so the vigor of plants from treated seed of this kind was considerably less than when the pericarp had been sound, or when the seed had been planted in damp soil at the start (Fig. 3).

Care was taken not to use seed that carried internal infection, so that isolations from the interior of the germ area indicated infections that had penetrated from the soil. In seed with the pericarp partially removed from the crown, isolations were obtained from a few kernels on the third day after planting in soil with 13 per cent moisture, and from nearly all kernels on the fifth day. These isolations consisted of *Trichoderma* species and an unidentified fungus (13). No *Pythium* species were isolated. The cause of seedling blight in dry soil has not been determined.

FIELD TESTS VERSUS COLD TESTS.—The cold test method obviously has great value for screening new fungicidal compounds, and some value for determining dosages, but it is not a substitute for field tests to obtain an indication of how much benefit in yield a farmer may expect from seed treatment. With a cold chamber one is not at the mercy of changing weather conditions and one can adjust the growing conditions sufficiently to cause serious seedling blight. In field tests in which seed with sound pericarps was planted during the first half of normal corn planting time, there was no stand increase from seed treatment. But when the same kind of seed was planted in similar soil and put into a cold chamber for 20 days at 50°F., the treatment resulted in striking increases in stand (Table 3). On the other hand, when hybrid seed with the pericarp partially removed from the crown and similarly treated with various fungicides (Table 3) was used for both field and cold tests, there was a highly significant correlation in stand of .687 between the 2 kinds of tests.

OBSERVATIONS ON FIELD TESTS.—Randomized plots 2 × 7 to 2 × 10 hills, spaced 39.6 in. apart (4,000 per acre) were used. Usually there were 10 replications of a seed composite or 5 replications of each of 2 or more hybrids in each seed treatment test.

*Time of planting.*—To obtain valid data on the value of seed treatments for commercial corn production, the planting should be made at a time consistent with normal agricultural practice for the area. Greater benefits from seed treatment are usually experienced when seed is planted abnormally early. This method has value for certain kinds of experiments, but the results are applicable only to those specific conditions.

*Rate of planting.*—In order to determine the benefits that may be expected from seed treatment in agricultural practice, the rate of planting should be consistent with recommended planting rates. The Agronomy Department, University of Illinois, recommends the planting of 4,000 seeds per acre for each 25 bu. yield expected per acre. This recommendation is based in part on data presented in Table 4. It is based not only on maximum yields during good growing seasons but, also on the fact that increased planting rates are frequently inversely correlated with standability of plants and grain quality, and the expectation of an occasional drought year. The seed treatment tests were planted at the rate of 12,000 and 16,000 seeds per acre, the higher planting rate being used on the more fertile soil. Planting at less than recommended rates may overemphasize differences in yield due to seed treatment.

*Stand in relation to yield.*—Several different relationships of stand to yield may arise in seed treatment tests. 1) No increase in stand or yield. This condition

TABLE 4.—*Comparative yields, average of 9 hybrids, at 5 rates of planting, and increases in yield for each increment of increased planting rate, on 2 soil areas that differed in productivity. Three-year average, 1951-1953, Urbana, Illinois*[a]

| Soil condition | Thousands of plants per acre | | | | |
|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 |
| Highly productive | | | | | |
| Stand increase for each increment, per cent | .... | 100 | 50 | 33 | 25 |
| Yield increase for each increment, per cent | .... | 62[b] | 22 | 10 | 1 |
| Yield, bushels per acre | 56[b] | 94 | 114 | 125 | 126 |
| Medium productive | | | | | |
| Stand increase for each increment, per cent | .... | 100 | 50 | 33 | 25 |
| Yield increase for each increment, per cent | .... | 54[b] | 7 | −14 | −9 |
| Yield, bushels per acre | 46[b] | 72 | 76 | 65 | 59 |

[a] These values were calculated from data by Pendleton et al, Table 18 (20), Table 10 (21) and Table 11 (22).
[b] Data for 1952 and 1953 only.

might conceivably arise under certain soil, temperature, and moisture conditions, but has not been experienced by the writer with the most favorable fungicides when the tests have been made with commercially processed seed. It is a common occurrence, however, when seed with a sound pericarp is used (Table 1). 2) Stand increase without increase in yield. This relationship may occur when normal germinating conditions in the spring are followed by drought so that the treated plots are more seriously overpopulated for the moisture available than the check plots. This condition was observed by the writer in 1936. Seed with the pericarp removed from the crown, and diplodia-infected seed, each gave a significant increase in stand from the use of New Improved Semesan Jr., Merko, and Barbak, the leading corn seed fungicides at that time, but there was no increase in yield. Due to drought the good seed check yielded only 15 bu. per acre. 3) There may be an increase in yield in the absence of an increase in stand. This condition probably is uncommon in the humid areas east of the Mississippi river, but it occurred in the Illinois tests in 1953. Emergence was retarded about 3 days by lack of moisture in the planting made May 19. This resulted in no significant increases in stand but some significance increases in yield from seed treatment (22). Reddy (24) pointed out, in connection with dry soil at corn germinating time in field tests in 1934 ". . . any condition militating against rapid germination gives greater opportunity for benefit from seed treatment." Unless emergence is checked by drought conditions for more than 10 days after planting, seedling disease, when seed is untreated, may reduce vigor greatly but with little damage to stand (Fig. 2, 3).

Finally, 4) there is the consideration of both increase stands and yields from seed treatment. The usual result in Illinois is that the percentage increase in yield is fully as much as the increase in stand (Table 1) (21, 22). In other tests it was found that with stand as the only variable (Table 4), when plant populations increased from 8,000 to 12,000, increase in yield on highly fertile soil was less than half as much as increase in stand, and at 16,000 plants, yield increases became still much less. On soil of medium fertility, yield increases from increased stand were still less or nonexistent (Table 4). This clearly indicates that the large yield increases from seed treatment are not purely the effect of increased stand. Therefore, much of the increase in yield must be from healthier, thriftier plants due to treatment. This conclusion had been pointed out earlier at a time when open-pollinated corn was used (5, 24) and is reemphasized here with hybrid corn. Using sweet corn seed, others have pointed out that the increase in stand from treatment was accompanied by an increase in average plant vigor (2, 4).

*Border effects.*—In randomized blocks planted with the same hybrid but containing a number of treatments including the untreated check, each represented once, the check is always adjacent to treated plots, assuming that the outside border rows also are planted with treated seed. Thus the check is subjected to greater border effect than other plots, if all treatments are effective. The need for outside border rows is taken for granted, but there is some question as to how much border effect exists between plots within the experiment when no border rows are used. There have been no results published from experimental work with corn applicable to Illinois conditions that will directly answer this question. The writer has obtained data (unpublished) in which the center and outside rows of 4-row plots were harvested separately. From these results as well as from the work of others (1, 10, 11) it was concluded that if the difference between the check and the better treatments is not over 10 per cent in stand or yield, or both, the border effect is negligible and can be ignored. When the difference between adjacent plots is as much as 20 per cent, however, it becomes quite likely that the border effect will be measurable. The writer, therefore, has used only 2-row plots for the usual seed treatment tests. In experiments in which, due to the nature of the material, differences greater than 10 per cent were anticipated 4-row plots were used, at least for the untreated check, and the center pair and outside rows were harvested separately.

DISCUSSION.—Tests designed to measure benefits obtained from seed treatments indicate that increases in yield are the usual experience. The benefit from seed treatment has increased rather than diminished during the last 2 decades. The change has come about because of more pericarp damage from the mechanization of seed harvesting and preparation procedures and the availability of better seed protectants for pericarp-damaged seed. Some of the nonmercurial chemicals are more effective not because they are better fungicides but because they reduce seed injury.

Hoppe (6) found that mercurial seed treatments were injurious to the seed when pericarp injuries occurred near the embryo.

As the field performance of seed corn with sound pericarps usually benefited very little, if any, from fungicidal seed protectants, and as commercially processed seed produced 10 per cent less stand and 10 per cent less yield than seed with sound pericarps when neither kind was treated, and as the latter responded markedly to treatment, the importance of considering the pericarp condition of seed selected for seed treatment experiments appears obvious. The results from seed treatment experiments with hand-handled seed with 100 per cent pericarp injury induced by laboratory manipulation more nearly approximate results from commercially processed seed than did those from seed with sound pericarps.

Part of the reason for immature seed being more susceptible to seedling blight is no doubt due to the fact that an appreciable number of kernels develop pericarp injury spontaneously during the drying process. The principal reason, however, must be looked for elsewhere but has not been determined. Dimmock (3) found that immature seed dried at 108°F. gave a greatly retarded and reduced total emergence as compared with similar seed air-dried. This may have been due, at least in part, to an increase in pericarp injury. He states: "In comparison with air drying, its only noticeable effect upon the appearance of the kernels was to cause a somewhat greater degree of blistering or lifting of the pericarp and a bleaching of colour in the immature seed."

In reporting results from cold tests it has frequently been mentioned that seed treatment was of only moderate value for increasing stands and vigor. In other cases very good results have been reported. It is probable that different species of fungi may be involved that differ in their resistance to the fungicide. The most usual reasons, however, appear to be differences in degree of infestation of the soil in relation to soil moisture and time of subjection to low temperature. Seed treatment with some fungicides provides excellent protection against pythium seedling blight up to a certain point, beyond which it becomes increasingly ineffective. Where this point lies depends on many factors which include not only the conditions of the cold test mentioned above, but also the seed condition with respect to age and other debilitating influences, pericarp condition, genetic constitution, and differences in dosage of the fungicide.

In cool, moist soil, *Pythium debaryanum* and related species were nearly always the first fungi to be isolated from the scutellar area of the embryo. With pericarp-injured seed in field tests as well as in cold tests, the pythium seedling blight disease has usually caused reductions in stand as well as vigor. The writer has observed no cases of reduction in stand without reduction in seedling vigor as measured by height of plants, average number of leaves per plant, or green weight per row. Thus impairment in vigor is the most constant effect of corn seedling diseases and this may or may not be accompanied by reduction in stand. This appears to be true not only with pythium seedling blight, but of all seedling blight diseases studied by the writer. The thought sometimes expressed that loss of stand from disease could be satisfactorily compensated by thicker planting has not proved valid. That weak plants during the early part of the growing period do not ordinarily overcome this handicap has been confirmed by a large number of yield tests.

The lapse of time from planting in the field to emergence is very useful information in interpreting the results from seed treatment experiments. In the north-central region temperature is usually the principal determining factor but soil moisture, virility of the seed, etc., sometimes influences rate of emergence. Delayed germination, regardless of the cause, tends to increase disease damage which often can be controlled by seed treatment. Therefore, information on days from planting to emergence is of value when reporting results.

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# AMINO-ACID COMPOSITION OF MONOSPORIDIAL CULTURES OF USTILAGO ZEAE OF DIFFERENT SEX[1]

J. E. DeVay

SUMMARY

Quantitative and qualitative differences were found in the free amino-acid content of cultures of monosporidial lines of *U. zeae* from different sex groups, but they were not correlated with sex groups. No marked differences were found in the bound amino-acid content of lines of different sex. Apparently there is no relationship between the bound and free amino-acids synthesized in artificial culture by lines of *U. zeae* and their sex and pathogenicity.

The biochemistry of sexual reproduction in *Ustilago zeae*, the common smut of corn, is apparently more complex than in many microorganisms. No combinations of naturally occurring substances and environmental conditions have been found which enable compatible lines of *U. zeae* to complete the life cycle outside the host plant. Furthermore, the knowledge that the species comprises an indefinitely large number of biotypes representing multiple sex groups (2, 9), suggests that the chemistry of sex is somehow related to the ability of these biotypes to produce a group of compounds which when present in proper concentrations determine sexual compatibility in host tissue.

The present investigation was made to find out whether there might possibly be an association between the formation of free or bound amino-acids by biotypes of *U. zeae* and their sex and pathogenicity.

BIOLOGICAL MATERIALS.—Thirty culturally different monosporidial haploid lines, isolated from smut galls collected in Ohio and New York, were selected after initial screening tests of their sexual compatibility. In addition, 2 haploids, 17D4 and 10A4, an amphisexual line, 410qq-8, and 2 solopathogenic lines, 410n and 410qq, obtained from the stock cultures of the Department of Plant Pathology, University of Minnesota, were also included in this study. All these lines were maintained in the sporidial growth phase by growing them on a medium containing 10 gm. of dextrose, 6 gm. of agar, and the broth from 300 gm. of boiled potatoes per liter.

The sexual potentialities of these lines were determined by pairing them in all possible combinations and inoculating corn seedlings by the partial vacuum method (6, 11). A smut-susceptible inbred line of dent corn, B-164, was used for all seedling inocula-

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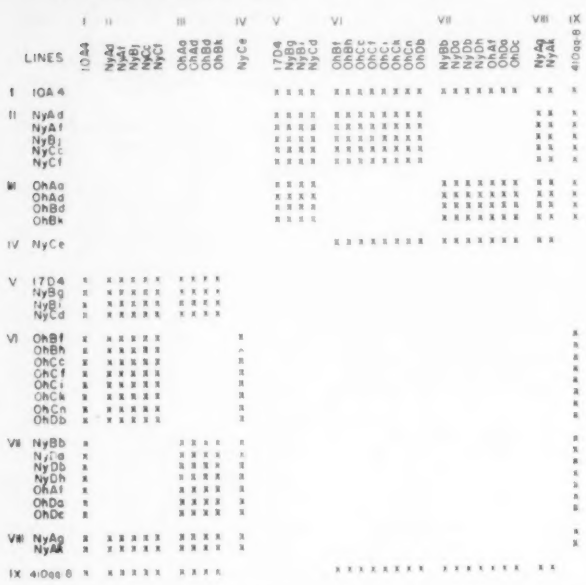
TABLE 1.—*Sexual and pathogenic compatibilities of haploid lines of Ustilago zeae* [a, b]


I II III IV V VI VII VIII IX
LINES
1 10A4
II NyAd NyAf NyBj NyCc NyCf
III OhAa OhAd OhBd OhBx
IV NyCe
V 17D4 NyBg NyBi NyCd
VI OhBf OhBh OhCc OhCf OhCi OhCk OhCn OhDb
VII NyBb NyCa NyDb NyDh OhAf OhDa OhDc
VIII NyAg NyAk
IX 410gg 6

[a] Roman numerals designate different sex groups and the x's represent compatible crosses in which galls and mature chlamydospores were formed.

[b] This table was arranged, for the most part, by Dr. J. B. Rowell.

tions. The results of these crosses are given in Table 1.

CULTURE METHOD.—Smut lines were grown in 500-ml. Erlenmeyer flasks containing 250 ml. of the following modified Pfeffer's medium (10): $Ca(NO_3)_2 \cdot 4H_2O$, 0.333 gm.; $MgSO^4 \cdot 7H_2O$, 0.083 gm; $KNO_3$, 0.083 gm.; $KH_2PO_4$, 0.083 gm.; KCl, 0.042 gm.; $FeCl_3 \cdot 6H_2O$, 0.24 mg.; $ZnCl_2$, 0.15 mg.; $H_3BO_3$, 0.06 mg.; $CuCl_2 \cdot 2H_2O$, 0.05 mg.; $MnCl_2 \cdot 4H_2O$, 0.04 mg.; $NaMoO_4 \cdot 2H_2O$, 0.03 mg.; dextrose, 10.0 gm.; and distilled water to 1 liter. The salts used in this medium were of chemically pure grade. The experimental flasks were capped with 2 layers of filter-paper held in place with rubber bands and autoclaved 30 minutes at 121°C. The flasks were then inoculated with 1-ml. amounts of sporidial cultures and put on a rotary-action shaker for 10 days to allow the cell populations to reach about 90–100 million cells per ml. If shaken longer, the cell populations would have increased to about 120–130 million cells per ml., accompanied by increasing autolysis and a decrease of free amino-acids in the cultures.

After being shaken for 10 days, the cultures were left stationary 3–10 days to increase the concentrations of free amino-acids. The rapid increase in free amino-acids during the stationary culture period was probably due to decreased culture aeration. In previous studies (6, 8), cultures of equal volumes of a monosporidial line, 410n, when grown in 3-l. Fernbach flasks and 500-ml. Erlenmeyer flasks on a rotary-action shaker, differed in their free amino-acids. The relatively deep cultures in Erlenmeyer flasks were aerated less than the shallow cultures in Fernbach flasks but contained more kinds and amounts of free amino-acids. The temperature during these growth periods varied from 25°–29°C. Non-inoculated flasks of culture medium served as controls.

CULTURE EXTRACTION.—In early experiments, cultures were frozen for 1 week at —25°C. before extracting them, but in later experiments they were autoclaved at the end of the growing period and 95 per cent ethanol was added to bring the alcohol concentration in each culture to 70 per cent to extract the alcohol-soluble nitrogen fraction. Cultures were extracted at room temperature (25–29°C.) for 24 hours and centrifuged. The clear, supernatant layers were then concentrated under reduced pressure at 50°C. to near dryness for each extract and made up to 10 ml. with 70 per cent ethanol.

Bound amino-acid determinations were made on cellular materials which had been extracted with 70 per cent ethanol, air-dried, and ground in a Wiley mill to pass through a No. 40 mesh sieve. The materials were then dried in an oven at 50°C. for 24 hours. Six-hundred mg. of each smut line were hydrolysed by boiling the cellular material for 24 hours in 40 ml. of 6-N hydrochloric acid solution under a reflux condenser. The HCl was removed by concentrating the hydrolysates to dryness under reduced pressure at 70°C. after which 10 ml. of water was added to each of the samples which were then reconcentrated to dryness. Residues were extracted with 10 ml. aliquots of 70 per cent ethanol and centrifuged to remove the insoluble material.

CULTURE ANALYSIS.—The concentrated extracts were analysed for ninhydrin-reacting compounds by paper partition chromatography (3), using essentially the method of Dent (4, 5). Special methods for the determination of histidine (7), cystine, and methionine (4) were also used. The extracts were spotted at the appropriate position on Whatman No. 1 filter-paper sheets (18″ × 22″) with a nichrome wire loop or 5-$\mu$l. pipettes. The concentration of an extract on a single spot was built up by allowing each application to dry completely on the paper before applying more extract. Prior to developing a chromatogram, the area in which the superimposed spots were located was exposed to ammonia vapor. The chromatograms were then irrigated for approximately 24 hours with phenol saturated with water in a closed cabinet containing ammonia vapor and a saturated atmosphere of phenol-water vapor, dried at room temperature for 24 hours and irrigated for 40 hours in a second direction with a mixture of 1 vol. 2,4-lutidine and 1 vol. 2,4,6-collidine saturated by adding 2 vol. of distilled water. Solvents were equilibrated and chromatograms were irrigated at 25–29°C.

After development, the chromatograms were air-dried for about 10 hours and then sprayed with a ninhydrin solution (0.4 per cent w/w triketohydrindene hydrate crystals and 1.0 per cent w/w phenol crystals dissolved in 70 per cent ethanol) to reveal the locations

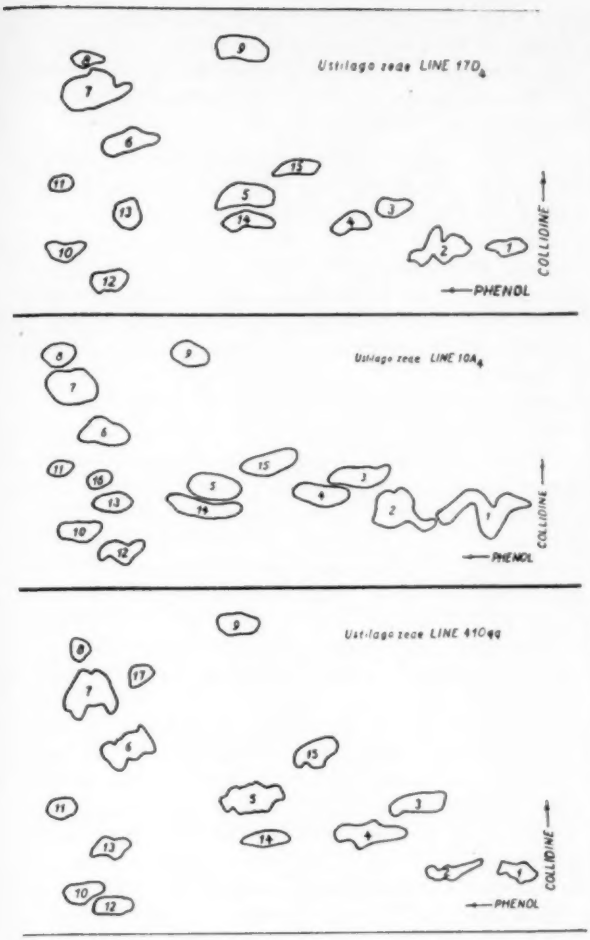



FIG. 1. Two-dimensional chromatograms of the free amino-acids in cultures of *Ustilago zeae*. Lines 17D4 and 10A4 are haploids of opposite sex whereas line 410qq is a solopathogen derived from them. 1, glutamic acid; 2, aspartic acid; 3, serine; 4, glycine; 5, alanine; 6, valine; 7, leucine and/or isoleucine; 8, phenylalanine; 9, tyrosine; 10, arginine; 11, proline; 12, lysine; 13, gamma-amino butyric acid; 14, glutamine 15, threonine; 16, unknown; 17, unknown.

of the ninhydrin-reacting compounds. The color intensity of the resulting spots was increased by heating the chromatograms in an oven at 85°C. for 5 minutes. The identity of the spots was established by comparing the $R_f$ values of the amino-acids in a known mixture with those in the culture extracts, by characteristic spot colors, and by superimposing known mixtures of amino-acids on the spots of the culture extracts before irrigating the chromatograms.

EXPERIMENTAL RESULTS.—Two-dimensional chromatograms of the free amino-acids in culture extracts of lines 17D4, 10A4, and 410qq are shown in Fig. 1. These chromatograms are representative of those obtained by chromatographing the free amino-acids in culture extracts of other lines listed in Table 1. Approximately 25 μl. of each extract were applied at the appropriate position on filter-paper sheets prior to develoment. The following free amino-acids were found in each extract: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, glutamine, lysine, arginine, gamma-amino butyric acid, proline, valine, leucine and/or isoleucine, phenylalanine, and tyrosine. The culture extract of haploid line 10A4 contained an unidentified ninhydrin-reacting compound, stable to acid hydrolysis, which was not found in the other extracts. In contrast, another unknown was found only in the culture extract from solopathogenic line 410qq.

Visual comparisons of chromatograms of the 17D4, 10A4, and 410qq extracts showed that each contained approximately the same concentration of alanine. These comparisons are based on the fact that size and color intensity of spots caused by the reaction between ninhydrin and certain compounds is largely a function of the concentrations of the compounds (5). Using the alanine spots as standards of comparison (Fig. 1), glutamic acid, aspartic acid, and glutamine were most concentrated in the 10A4 extract whereas the concentration of aspartic acid in the 17D4 extract was apparently greater than that in the 410qq extract. These were the most marked quantitative differences although other differences may have been found if more refined measurements of amino-acid concentrations had been made (1). Methionine, cystine, and histidine were not found in the extracts of free amino-acids.

Because the cellular weights (air-dried) of lines 17D4, 10A4, and 410qq were 0.21, 0.32, and 0.48 gm., respectively, the quantitative and qualitative differences observed could have been due to differences in growth rate rather than to differences in sex or other physiologic characters. However, cultures of lines 17D4 and NyBb had approximately the same dry weight but did not have the same culture pH (Table 2) or a similar free amino-acid content (Fig. 2) whereas cultures of lines NyBb and NyBg had different cellular weights but had a similar pH and free amino-acid content.

Fig. 2 shows a series of 1-dimensional chromatograms of the free amino-acids in culture extracts of 6 lines representing 2 sex groups. Twenty-five μl. of a concentrated culture extract of each line were spotted in the designated places (x) on the chromatograms prior to irrigation with phenol saturated with water for 24 hours at 25–29°C. Lines representing sex group V (Table 1) are under A whereas those of sex group VII are under B. Comparisons of the chromatograms under A show quantitative and qualitative differences; like-

TABLE 2.—*Dry weight and final pH of the cultures of lines in Fig. 2*

| Line | Dry weight | Final pH |
|---|---|---|
| NyCd | 0.10 gm. | 5.78 |
| NyBg | 0.44 | 4.91 |
| 17D4 | 0.13 | 6.00 |
| OhAf | 0.44 | 6.60 |
| NyDb | 0.17 | 6.30 |
| NyBb | 0.12 | 4.92 |

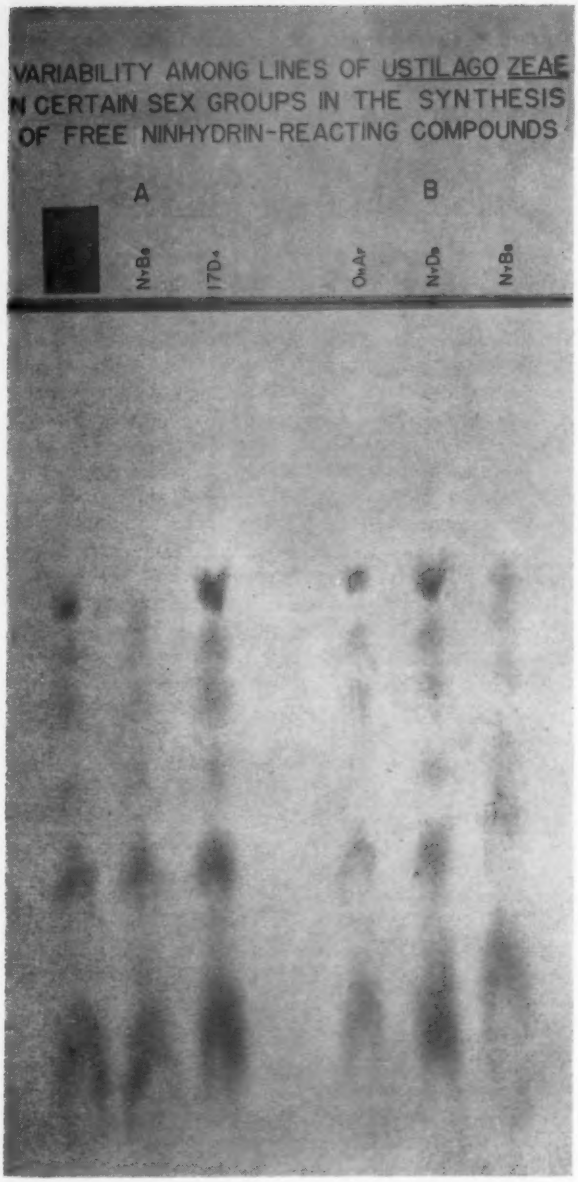



FIG. 2. One-dimensional chromatograms of the free amino-acids in culture extracts of 6 lines of *Ustilago zeae* from 2 sex groups. A) Lines representing sex group V. B) Lines representing sex group VII (Table 1).

wise, comparisons of chromatograms under B show similar differences. However, the chromatograms of lines 17D4 and NyDb are similar as are the chromatograms of lines NyBg and NyBb. Thus, there was as much variability in the synthesis of free ninhydrin-reacting compounds among lines from the same sex group as among lines from different sex groups.

Because no correlation was found between the sexual potentiality of a line of *U. zeae* and its ability to accumulate different kinds and amounts of free amino-acids in artificial culture, the work was extended to include bound amino-acid determinations. Analyses were made on cellular materials of monosporidial lines which had been extracted with 70 per cent ethanol and acid-hydrolysed. Thirty $\mu$l. of each extract were spotted on filter-paper sheets using 5-$\mu$l. pipettes and then developed as previously described.

Chromatograms of the bound amino-acids in cultures of lines NyCd, NyBg, and 17D4 of sex group V are shown in Fig. 3. Visual comparisons of these chromatograms and of other lines studied revealed no marked differences in the bound amino-acid content of lines of *U. zeae*. Furthermore, the chromatograms of solopathogenic lines 410n and 410qq, and an amphisexual mutant of 410qq, line 410qq-8, were similar to those in Fig. 3. Thus, no relationship was found between the sex and bound amino-acid content of these lines.

Bound amino-acids found in the cultures of *U. zeae* were: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, arginine, proline, valine, leucine and/or isoleucine, tyrosine, cystine, methionine, histidine, and 2 unknowns. One unknown, spot 13, corresponded in position to gamma-amino butyric acid, while the other unknown, spot 18, corresponded in position to homocystine (5). This last unknown was not found in any of the extracts of free amino-acids. Cystine and methionine were found by means of a special method (4) in which the extracts were treated with hydrogen peroxide before being chromatographed. Histidine was identified with a special method (6, 7) in which 2 ml. of a culture extract were mixed with 4 ml. of 4 per cent sodium carbonate solution and then treated with 0.1 ml. of diazotized p-nitroaniline. The resulting azo dyes were extracted in 2 ml. of n-butanol and spotted on filter-paper strips. The chromatograms were then irrigated for 48 hours with s-butanol saturated with water at 17°C., air-dried, and sprayed with a 4 per cent solution of sodium carbonate to intensify the color of the dyes.

DISCUSSION.—In earlier work it was found that haploid lines of *U. zeae* grown in a synthetic medium would produce some substance or substances that can increase the virulence of certain other haploid lines and possibly in some instances enable the completion of the life cycle (6). The chemical nature of this substance is largely unknown, but the fact that *U. zeae* can synthesize from a solution of mineral salts and a single carbon source, such as glucose, all the compounds it needs for growth, and the fact that numerous mating types exist, indicates that the compounds controlling sex and pathogenicity in this species may have a similar basic structure, certain modifications of which may be synthesized by lines of different sex. The high degree of specificity in the sexual compatibilities of various mating types could be controlled by compounds such as amino-acid peptides or proteins.

No amino-acid peptides were found in cultures of *U. zeae*, although compounds of this type may have been present in concentrations too low to detect with the methods used. It was found that lines that differed in sex and pathogenicity also differed in the free

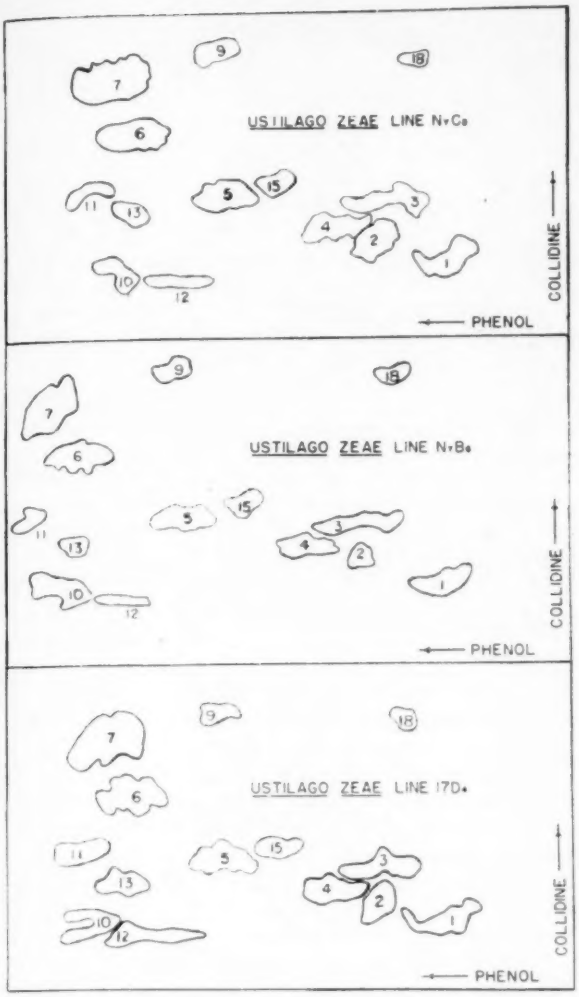



FIG. 3. Two-dimensional chromatograms of the bound amino-acids in cultures of haploid lines of *Ustilago zeae*. 13, an unknown corresponding in position to gamma-amino butyric acid; 18, an unknown corresponding in position to homocystine.

amino-acid content of their cultures, although there is no apparent relationship between these differences and the sexual potentialities of the lines.

The fact that mating types in *U. zeae* are not reflected in various amino-acid analyses, does not eliminate the possibility that amino-acids or combinations of them control sexual compatibility in host tissue. An "in vitro" study such as this is handicapped because of the influence of an artificial environment on cell metabolism which in turn may eliminate or lessen important differences characteristic of different mating types in the host. Studies are now being made to determine serological relationships of lines among and within different sex groups of *U. zeae*.

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# BACTERIA AND FUNGI IN SEEDS AND PLANTS OF CERTIFIED BEAN VARIETIES [1]

William C. Schnathorst [2]

SUMMARY

Isolations were made from seeds and plants of 4 varieties of Idaho certified bean.

Twenty-three bacterial and 8 fungal isolates were obtained. Of the bacteria identified, *Bacillus cereus, B. megatherium, B. subtilis,* and *Pseudomonas* sp. were found in seeds only; *Achromobacter* sp., *Aerobacter aerogenes, Alcaligenes viscosus, Bacillus cereus* variation, *B. polymyxa,* and *B. sphaericus* were found to exist in seeds and plant tissues; and *Aerobacter cloacae, Agrobacterium radiobacter, Alcaligenes* sp., *Bacterium globiforme, Corynebacterium helvolum, Micrococcus,* sp., and *Pseudomonas fluorescens* were found in or on plant tissues only. Concerning the fungi, 4 strains of *Rhizoctonia solani* were isolated from seeds, Strain No. 1 being the only 1 of the 4 isolated from tissue. *Alternaria* sp., *Fusarium* sp., and *Stemphylium* sp. were also isolated from seeds. *Sporotrichum* sp. was isolated from plant tissue.

The genus *Bacillus* was by far the most frequent group of organisms found in both seeds and tissues. The genera *Aerobacter* and *Alcaligenes* and species *Bacterium globiforme* and *Agrobacterium radiobacter* were often isolated. The remaining isolates ranged from several isolations to only 1. *Rhizoctonia solani* was the most prevalent fungus.

None of the bacteria were detectably pathogenic to *Glycine max, Melilotus officinalis, Phaseolus lunatus* var. *limenanus, P. vulgaris* var. Black Valentine, or *Vigna sinensis* var. Blackeye. *Sporotrichum* sp. and 4 strains of *Rhizoctonia solani* were tested for pathogenicity. All were pathogenic to the above plant species.

The evidence indicates that bacteria are not present in embryonic tissues, but are common under the seed coat of bean seeds.

Appearance of bean blights in Wyoming incited by *Xanthomonas phaseoli* (Smith) Dowson, *Pseudomonas phaseolicola* (Burk.) Dowson, and *Corynebacterium flaccumfaciens* (Hedges) Dowson, on plants grown from certified bean seed in isolated fields and in soil believed to be free from causal organisms has caused much concern about purity of the seed. Investigations were undertaken to ascertain the organisms present in seed and plants of certified stock and their effect on bean plants. Information on existence of organisms in apparently healthy plant tissues will aid in better understanding of disease epidemics, adaption of pathogens to new hosts, and disease resistance.

Early papers treating the presence of microorganisms in healthy tissues were reviewed by Smith (19). In a few cases microorganisms were obtained, but many isolations were attributed to expected contaminants. Consequently the belief that healthy tissues were free of microorganisms was not seriously disputed until recent times. Fifteen papers (1, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 20, 21, and 22) since 1926 reported the presence of bacteria in apparently healthy plant tissues and 7 papers (2, 10, 11, 12, 15, 16, and 22) since 1939 have reported fungi existing in healthy tissues.

MATERIALS AND METHODS.—Isolations were made from seed and plants grown from seed of 2 varieties of Great Northern, University of Idaho (U. I.), Nos. 16 and 123, and 2 varieties of Pinto U. I., Nos. 78 and 111, certified in Idaho in 1950. Plants were grown in steam-sterilized soil in pots on clean wooden benches in greenhouses kept at 75°F.

Seeds selected for isolations were rinsed in tap water, dipped in 95 per cent alcohol, immersed in 0.2 per cent mercuric chloride for 30 seconds, and rinsed with 10 ml. of sterile distilled water. Tissues selected for isolations were, except where otherwise stated, rinsed in tap water, dipped in 95 per cent alcohol, placed in 0.1 per cent mercuric chloride for 3 minutes, and rinsed with 10 ml. of sterile distilled water.

Instruments used in handling seeds and tissues were stored in alcohol and thoroughly flamed before use. All culture work was done in a transfer chamber, the working surface of which was washed with 0.2 per cent mercuric chloride and covered with a dampened piece of cheesecloth. Organisms obtained were incubated at 26 ± 2° C.

Bacterial isolations were made from seed selected. unless otherwise stated, by randomized sampling. Where macerated seeds were used, they were soaked in sterile distilled water for 24 hours and broken up with a setrile glass rod. Serial dilutions up to 1:1,000 were made from the suspension. Cultures were made by pipetting 1 ml. of each suspension and then adding Burkholder's medium which consists of the following, grams/liter: potato, 300; peptone, 5; sodium phosphate, 2; sodium chloride, 2; sodium citrate, 1; dextrose, 6; agar, 17; and asparagine, 1.

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Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming No. 225.

[2] Graduate Assistant, Division of Plant Pathology, University of California, Davis, California. The author acknowledges the guidance of Drs. H. J. Walters and W. G. Solheim of the University of Wyoming. Thanks are due Mr. G. W. Robertstad, University of Wyoming, for verifications of most of the bacteria found. The author expresses appreciation to Drs. W. W. Diehl and W. J. Zaumeyer, United States Department of Agriculture, Beltsville, Maryland, for identification of 2 fungi isolated.

Isolations of bacteria from the embryos and seed coats were attempted by placing soaked seeds on a pad-like piece of cheesecloth, previously saturated with 0.1 per cent mercuric chloride solution, which in turn rested on the bottom of a moisture chamber dish. The seed coat, cotyledon, radicle, hypocotyl, and epicotyl portions of seeds were removed and individually placed in plates of potato-dextrose agar and in tubes of trypticase soy broth. Trypticase soy broth consists of the following, grams/liter: trypticase, 17; phytone, 3; sodium chloride, 5; dipotassium phosphate, 2.5; and dextrose, 2.5.

Bacteria and fungi were isolated from discolored and shriveled seeds by placing individual seeds on potato-dextrose agar in culture dishes to which were added 5 ml. of sterile distilled water to allow germination of the seed and growth of organisms through the softened seed coat.

Microorganisms were isolated from plant tissues by 2 techniques. The first involved taking various tissues from plants of different ages grown from normal appearing seed. Tissues were taken from plants with sterilized equipment, passed through 3 sterile distilled water rinses, placed in 10 ml. of sterile distilled water, and then broken up. Dilutions of the suspension were made and plates poured as was done with crushed seeds. The second technique involved quartering 4-in. sections of stems of plants grown from discolored and shriveled seed. After surface-sterilization, 2 quarters were cultured per plate of potato-dextrose agar.

Anaerobic isolations were made by embedding plant tissues in 65 ml. of Brewer anaerobic agar (3) in a culture plate and covering the dish with a Brewer anaerobic Petri dish cover.

To obtain pure bacterial cultures, dilution plates were made and portions of different colonies transferred to tubes. Individual isolates were observed as culture streaks, smears, stabs, and as colonies on Burkholder's medium, potato-dextrose agar, and nutrient agar (3). Abundance of growth, consistency, depth, chromogenesis, size, shape, and surface appearance of cultures were studied. Duplicate cultures of similar isolates were discarded; those remaining were numbered and subjected to at least 22 additional determinative tests as recommended in the Manual of Methods for Pure Culture Study of Bacteria, issued by the Society of American Bacteriologists. *Escherichia coli* (Migula) Castellani & Chalmers was used as a control for most of the tests.

Organisms isolated were subjected to pathogenicity tests involving the following species of legumes: *Glycine max* Merr., *Melilotus officinalis* Lam., *Phaseolus lunatus* var. *limenanus* Burkhart, *P. vulgaris* L. var. Black Valentine, and *Vigna sinensis* Savi var. Blackeye. Bacterial inoculations were made with a Cornelius Hydraulic Aerosol sprayer using a suspension containing approximately 15 million viable bacteria per ml. Each of the primary leaves of 3 plants per pot, except with *Melilotus officinalis*, were inoculated with approximately 2 ml. of the desired suspension when the plants were about 20 days old. With *M. officinalis*, approximately 20 leaflets per pot were inoculated with approximately 0.25 ml. of suspension per leaflet when the plants were 2 to 3 months old.

Fungal isolates were grown on potato-dextrose agar. Broken-up agar and mycelium from 1 Petri plate culture were placed on steam-sterilized sand in a quart jar. Ten surface-sterilized seeds of the desired plant species, 100 of *M. officinalis*, were placed on the fungal layer in each jar and a layer of sterilized sand added. Contents of each were moistened with distilled water and each jar was covered with cellophane held in place with a rubber band (4).

EXPERIMENTAL RESULTS. — *Bacteria from seeds.* — Eleven species of bacteria were isolated from 40 crushed surface-sterilized bean seeds all of which yielded bacteria. Most species apparently existed in seeds as a mixed flora which was demonstrated by their presence on plates prepared by serial dilution. According to number, the isolates were as follows: 3, 5, 8, 9, 10, 11, 12, 17, 18, 19, and 21 (Table 1).

To verify presence of bacteria in seeds, 41 seeds were cultured in trypticase soy broth, 1 seed per tube. Bacteria appeared in all tubes. No attempt was made to identify these bacteria, but cultural comparisons with bacteria isolated by crushing of seeds showed them to be similar and to occur in a mixed flora.

To ascertain more precisely the location of bacteria in seeds, isolations from the seed coat and radicle, hypocotyl, epicotyl, and cotyledon portions of the embryo were attempted. Individual portions of seeds

TABLE 1.—*Identification of bacterial isolates obtained from seeds and tissues of beans*[a]

| Culture No. | Identification Genus and species |
|---|---|
| 1 | Not identified |
| 2 | *Alcaligenes* sp. Castellani & Chalmers.[b] |
| 3 | *Pseudomonas* sp. Migula.[b] |
| 4 | *Pseudomonas fluorescens* Migula. |
| 5 | *Aerobacter aerogenes* (Kruse) Beijerinck. |
| 6 | *Corynebacterium helvolum* (Zimmerman) Kisskalt & Berend. |
| 7 | *Micrococcus* sp. Cohn.[b] |
| 8 | *Alcaligenes viscosus* (Weldin & Levine) Weldin. |
| 9 | *Achromobacter* sp. Bergey et al.[b] |
| 10 | *Bacillus subtilis* Cohn *emend.* Prazmowski. |
| 11 | *Bacillus cereus* Frankland & Frankland. |
| 12 | *Bacillus megatherium* De Bary. |
| 13 | Not identified |
| 14 | *Bacterium globiforme* Conn. |
| 15 | Not identified |
| 16 | *Agrobacterium radiobacter* (Beijerinck & van Delden) Conn. |
| 17 | *Bacillus cereus* variation Smith, Gordon, & Clark. |
| 18 | *Bacillus polymyxa* (Prazmowski) Migula. |
| 19 | *Bacillus sphaericus* variation Neide. |
| 20 | *Aerobacter cloacae* (Jordan) Bergey et al. |
| 21 | Not identified |

[a] Identifications taken from Bergey's Manual of Determinative Bacteriology, 6th edition.
[b] The authority given is for the genus only.

were removed, surface-sterilized and placed on potato-dextrose agar and in trypticase soy broth. Eighteen seeds were tested, 9 by each method. Bacteria were obtained from cotyledons of 2 seeds placed on agar and from cotyledons of 1 seed cultured in broth. Negative results were obtained from seed coats, epicotyl, hypocotyl, and radicle portions. Eight broth tubes containing cotyledons and 3 containing seed coats exhibited presence of gas, but 3 attempts at isolation proved the absence of organisms.

*Bacteria and fungi in seeds.*—To determine the presence of microorganisms in bean seed, 30 discolored and shriveled and 14 normal appearing seeds were individually cultured for 14 days in culture plates on potato-dextrose agar to which 5 ml. of sterile distilled water were added. Sixty-five per cent of the discolored seeds yielded bacteria or fungi and 21.5 per cent of the normal appearing seeds yielded only bacteria. Due to the extreme mixture of flora, no attempt was made to reisolate bacteria, but 6 fungi, Nos. 2, 3, 4, 6, 7, and 8, were obtained (see section concerning identification of fungi). Fungal isolate No. 1 was obtained from the seed coat of 1 dissected bean cultured on potato-dextrose agar.

*Bacteria and fungi in plant tissues.*—Isolations were made from leaf and stem tissue of apparently healthy plants and from "snakehead"[3] plants at various stages of growth after crushing in sterile distilled water. Bacteria were present in or on all of 40 tissue pieces sampled. Ten distinct isolates were obtained and assigned numbers as follows: 1, 2, 4, 5, 6, 7, 8, 9, 22, and 23 (Table 1). Isolates 22 and 23 were lost in culture.

Isolations also were made from plants grown from discolored and shriveled seeds along with normal appearing seeds by quartering 4-in. pieces of stems and placing 2 tissue pieces in each plate of potato-dextrose agar. Cultures were incubated 14 days and distinct colonies transferred to slants. Of 220 stem pieces sampled 49.5 per cent yielded bacteria and 1.4 per cent yielded fungi. Nine bacterial isolates, Nos. 13–21 (Table 1), were obtained from 63 original isolates. Only 2 fungi, Nos. 1 and 5, were obtained (see section on identification of fungi). No difference in frequency nor in type of organisms was observed between Pinto and Great Northern varieties of bean or between normal and "snakehead" plants.

Positive results for presence of bacteria were obtained with 24 tissue pieces on anaerobic medium. As with other cultures a mixed bacterial flora occurred.

[3] Seedling bean which has failed to develop primary leaves.

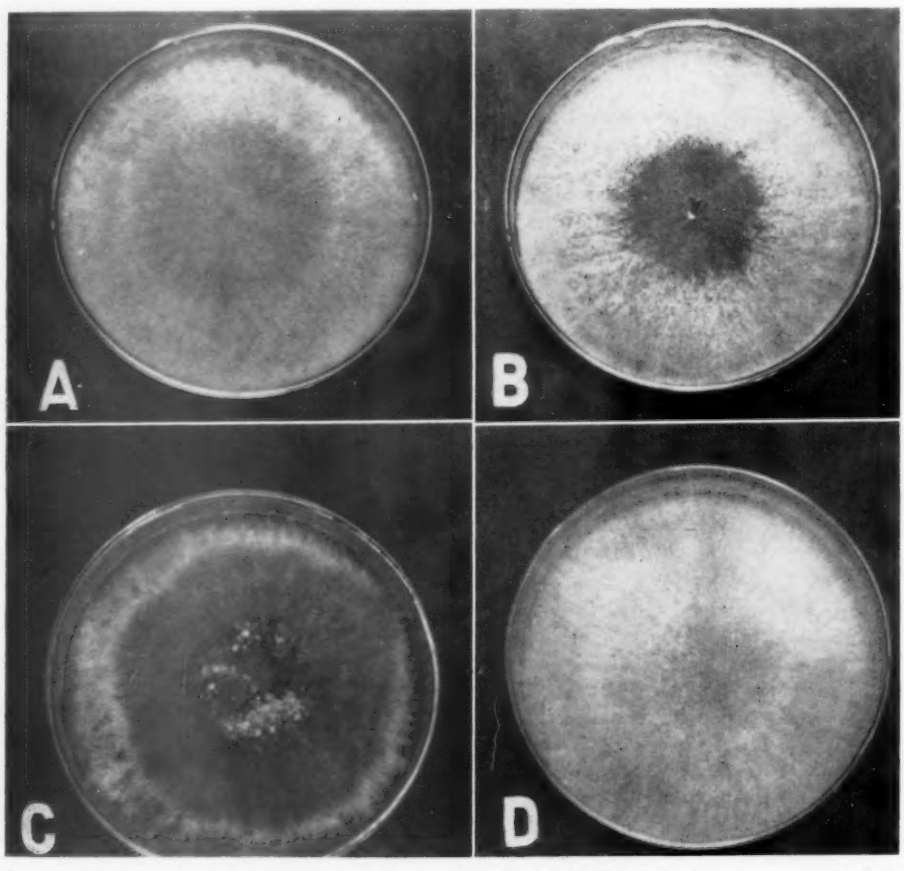


FIG. 1. Cultures of Strains 1, 2, 3, and 4 of *Rhizoctonia solani* A, B, C, and D respectively.

*Identification of bacteria.*—Identification of bacterial isolates, after determinative tests had been completed, was carried out to at least the genus, where possible, using Bergey's Manual of Determinative Bacteriology, 6th edition. Characteristics of each isolate can be found in thesis manuscript (18). Identifications of isolates are in Table 1.

Pathogenicity studies consisted of inoculating 5 species of legumes with each bacterial isolate. Following inoculation and after being in a moist chamber for 24 hours, plants were placed on clean wooden benches in greenhouses kept at 65° and 75° F. and leaves sprinkled several times daily for 3 weeks to keep up humidity. Adequate control plants were included. Negative results were obtained with repeated inoculations with all bacterial isolates. Positive results were obtained with *Xanthomonas phaseoli* inoculated to beans as a check at 65° and 75° F.

*Identification of fungi.*—Eight isolates were obtained from seeds and plant tissues and each given a number. Isolates, Nos. 6, 7, and 8, were identified from the original cultures. Isolates, Nos. 1, 2, 3, 4, and 5, were cultured on potato-dextrose agar for study of mycelium characteristics and chromogenesis. Microscopic studies were made on characteristics of hyphae and conidia. Optimum growth tests were also conducted. Identifications according to isolate number are as follows: Nos. 1–4 are strains of *Rhizoctonia solani* Kühn (Fig. 1); No. 5, *Sporotrichum* sp. Link; No. 6, *Alternaria* sp. Nees.; No. 7, *Stemphylium* sp. Walroth; and No. 8, *Fusarium* sp. Link. Characteristics of the fungi can be found in thesis manuscript (18).

Pathogenicity tests were conducted with isolates Nos. 1–5. Control jars were prepared in a manner similar to that used for test jars except that the medium contained no fungus growth. Each experiment included 2 test jars and 1 control for each plant species. Plants were grown approximately 14 days. Isolates, Nos. 1 and 5, were tested 3 times at 75° and 85°F. while isolates, Nos. 2, 3, and 4, were tested only once at 75° F.

All 4 strains of *Rhizoctonia solani* (isolates, Nos. 1, 2, 3, and 4) were pathogenic in varying degrees to all test species. Pathogenicity was characterized by complete rotting of seeds, dark brown lesions on stems at or near the soil line, and leaf blight consisting of necrotic tissue ranging from small circular spots to large rotted areas.

*Sporotrichum* sp. (isolate No. 5) was pathogenic at 75° and 85° F. to all test species.

Discussion.—Results of this investigation definitely support findings that microorganisms are present in apparently healthy plant tissues.

Those isolates which were found in seeds, as well as in surface-sterilized tissues, apparently comprise part of the true bacterial flora of bean plants.

Results obtained in isolating from seed coats and embryonic portions of seeds indicate that bacteria are not present in embryonic tissues, but exist under the seed coat. This agrees with the findings of Zaumeyer (23). The low percentage of isolations from cotyledons when compared to the 100 per cent yield from non-dissected seeds indicates that bacteria were under the seed coat and were killed by surface sterilization of parts dissected from seeds. When intact seeds were surface-sterilized the mercuric chloride contacted only the outer surface of the seed coat. Absence of bacteria from seed coats and the hypocotyl, epicotyl, and radicle portions of the embryo further substantiate this viewpoint.

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# THREE BACTERIA PATHOGENIC ON HEAD LETTUCE IN NEW YORK STATE[1]

Walter H. Burkholder

SUMMARY

Rots of head lettuce in New York State have been found to be caused by *Pseudomonas cichorii*, *P. marginalis* and *Xanthomonas vitians*. The cultural and physiological characteristics of the 3 pathogens set forth here compare favorably with the original and with amended description of the bacteria. Both species of *Pseudomonas* reduce nitrates to nitrites in a synthetic nitrate solution but frequently give a negative test in a nitrate broth. *P. marginalis* differs from descriptions of *P. aeruginosa* and is held to be a valid species. The 2 species of *Pseudomonas* have the ability to rot potato tubers, onion bulbs, and cabbage heads. *P. cichorii* also can cause a leaf blight of iris, and a wilt of bean seedlings. *P. intybi* is considered to be a synonym of *P. marginalis*, and *X. lactucae* and *X. lactucae-scariolae* synonyms of *X. vitians*. A similarity was found between *P. cichorii* and *P. viridiflava*.

The rotting of head lettuce in fields of New York State has occurred for many years. Bottom rot caused by *Rhizoctonia solani* and lettuce drop caused by *Sclerotinia spp.* are 2 rots readily diagnosed. Tip burn, a disease caused by unfavorable weather conditions not too well understood also results in a rot. Besides these diseases there are rots of unknown origin that have never been carefully studied in this region. The investigation reported in this article is an attempt to determine the cause or causes of these rots, study the pathogens and determine their relationships with lettuce pathogens previously described. In these studies, 3 bacterial pathogens have been found infecting head lettuce in the fields of New York State, and causing most of the unidentified diseases. The result of the infection by each bacterium is a rot so similar in appearance that it is difficult to distinguish one from another. The percentage of heads infected by the 3 pathogens varies with the fields, but at times the loss reaches ½ the crop or more. Fields having such high percentage of infection are scattered; nevertheless, the total loss each summer caused by the various bacteria becomes of considerable economic importance. Destruction of heads occurs mainly in the fields, but light and unnoticeable infection may lead to a rot in cut lettuce during transportation.

The pathogens involved are known species and have been described heretofore as causing diseases of lettuce and related crops. They are: *Pseudomonas cichorii* (Swingle) Stapp, *P. marginalis* (Brown) Stevens, and *Xanthomonas vitians* (Brown) Dowson. Miss Brown (2) in 1918 described *P. marginalis* and *X. vitians* and the diseases of lettuce they produce. Paine and Branfoot (12) also found *P. marginalis* to be pathogenic on lettuce in England. Later in the same laboratory Mehta and Berridge (11) stated that *P. marginalis* was identical with *P. aeruginosa* (*B. pyocyaneus*). Descriptions of this latter bacterium in Bergey's Manual (1) states that this species produces pyocyanin, has a high temperature range (optimum 37°C.), becomes brown in broth, and has the ability to ferment lactose and maltose; 4 characteristics not possessed by *P. marginalis*. These characteristics were not studied by Mehta and Berridge. Clara (4) pointed out 2 of these properties, pyocyanin production and lactose and maltose fermentation, as differences between *P. aeruginosa* and *P. polycolor*. Elrod and Braun (8) later stated that *P. aeruginosa* and *P. polycolor* are identical but made no careful study of the pathogens in culture. The fact that *P. aeruginosa* produces necrotic spots on lettuce and weakened tobacco leaves under moist conditions can not be used as a basis for considering the 3 species identical. Friedman (9) more recently gave a description of *P. marginalis* and the disease it produces on witloof chicory (*Cichorium intybus* L.). He considered *P. marginalis* a valid species and distinct from *P. aeruginosa*. Friedman also discussed the literature on these various lettuce diseases caused by species of *Pseudomonas* and showed that they are more or less world wide in distribution.

*Pseudomonas cichorii* was reported in 1925 by Swingle (14) in an abstract, and by Thornberry (16) in a brief note in 1927. *P. endiviae* described by Kotte (10) in Germany and *Bacterium formosanum* by

[1] Accepted for publication May 19, 1954.

Okabe (13) in Formosa are considered synonyms of *P. cichorii* (1).

Other species of bacteria that cause diseases of lettuce, chicory, endive, and related plants and that are listed by Miss Elliott (7) are *Pseudomonas intybi* (Swingle) Stapp, *P. rhizoctonia* (Thomas) Stevens, *P. viridilivida* (Brown) Holland, *Xanthomonas lactucae* (Yamamoto) Dowson and *X. lactucae-scariolae* (Thornberry and Anderson) Savulescu. Miss Elliott also reports that *P. aptata* (Brown and Jamieson) Stevens and *P. syringae* van Hall are pathogenic on lettuce. Furthermore, it is well known that *Erwinia carotovora* (Jones) Holland and related soft rot species can produce a rot of this crop.

Many references in literature to some of the bacteria that infect lettuce are not trustworthy since the rots are so similar in symptoms as to be indistinguishable, and only occasionally have the pathogens been isolated from the lesions and determined.

During the summer of 1951 and 1952 many infected heads of lettuce were examined and 17 isolates able to cause a rot of this crop were obtained. Five isolates proved to be *Pseudomonas marginalis*, 9 proved to be *P. cichorii* and 3, *Xanthomonas vitians*. This ratio probably is not found throughout the state since the 3 isolates of *X. vitians* came from only 1 county. The other 2 species showed a more even distribution. The bacteria in all instances were isolated from young lesions on the veins. When the disease progresses very far in the heads the leaves become rotten and slimy, and secondary organisms dominate and make isolations of the casual agent difficult or impossible.

Young lesions at times appear to be edematous and frequently show fissures. It was not ascertained, however, whether the pathogens caused this hypertrophy or whether the edema arose from other causes, developed cracks, and allowed the pathogens to gain entrance. *Pseudomonas marginalis* was obtained 3 times from rather yellowish lesions and *P. cichorii* 4 times from brown lesions. The difference in color, however, might have been due to age of the spots. *Xanthomonas vitians* definitely caused a darker spot than the other 2 pathogens.

During the course of this investigation, *Erwinia carotovora* was not found as a pathogen of lettuce. In 1945, however, it was isolated from a diseased head of lettuce where it was invading the vascular system of the plant and was associated with a species of *Bacillus*. The head of lettuce showed no rot, but was very pallid in appearance. *E. carotovora*, when isolated, rapidly produced a rot of lettuce upon artifical inoculation.

Tip burn of lettuce, especially in advanced stages, is difficult to distinguish from the bacterial rots. In the early stages, the disease may be more readily diagnosed. Beginning symptoms are a necrosis of the edges of the inner leaves that may be dry but are more often wet and slimy. At first, bacteria can not be found in these lesions but later the dead tissue may be invaded by undetermined bacteria. A considerable rot then ensues.

INOCULATIONS. — All the isolates of *Pseudomonas marginalis*, *P. cichorii*, and *Xanthaomonas vitians* when inoculated into head lettuce by smearing bacteria from agar cultures over cut heads placed in moist chambers caused infection and developed a rot. The rot produced by the 2 species of *Pseudomonas* was soft but that induced by *X. vitians* was not so slimy. In the greenhouse lesions caused by *X. vitians* on stems and veins of young plants were definite but decidedly not a wet rot. Old plants a month after infection sometimes showed a rotting probably favored by a moist condition or induced by secondary organisms.

Inoculation experiments also were conducted on lettuce in the greenhouse with an isolate of *X. lactucae-scariolae* received from H. W. Anderson. Two weeks after inoculation there appeared lesions that spread on the veins and into the leaf tissue (Fig. 1). Similar lesions resulted when inoculations were made with *X. vitians*.

Since *Pseudomonas marginalis* has been stated (6) to cause a rot of potato tubers, all isolates of the 3 pathogens were inoculated into tubers and kept in moist chambers. The 2 species of *Pseudomonas* rotted slices of tubers only a little less rapidly than did *Erwinia carotovora* or *E. atroseptica*. No rot, however, developed when tubers were inoculated with *Xanthomonas vitians*. Furthermore, *P. cichorii* and *P. marginalis* have the ability to rot onion bulbs and cabbage heads when inoculated in the same manner as the potato tubers. The former produces a very definite rot on these hosts and the latter light to fair lesions. Recently a species of *Pseudomonas* was isolated from a naturally infected head of cabbage taken from storage. The organism was pathogenic and appeared to be *P. marginalis*.

Inoculation experiments also on young iris plants and on bean seedlings with 2 isolates of *P. cichorii* and 3 isolates of *P. marginalis* gave infection with only the former. With iris, streaks appearing on the leaves extended several inches above the inoculation point; death of the leaf ensued. Little rot appeared on the rhizomes. The lesions on these infected leaves might easily be mistaken for those caused by *Erwinia carotovora*. It should be noted that 3 species of *Pseudomonas* in the past have been described as pathogenic on iris. They are *P. iridicola* (Takimoto) Stapp, *P. fluorescens exitiosa* van Hall and *P. iridis* van Hall (7). None of these 3 species has been described adequately so it would be difficult to state their relationship to *P. cichorii*. *P. iridicola*, the best described of the 3, does not agree with *P. cichorii* in its action on gelatin and in milk.

Bean seedlings, inoculated by stabbing the stem with a scalpel contaminated with a culture of *P. cichorii*, rapidly wilted. The fact that this organism and *P. viridiflava*, a bean pathogen, have many cultural characteristics in common leads to the possibility that they are identical species. Extensive work, directed toward elucidation of their relationship should, how-

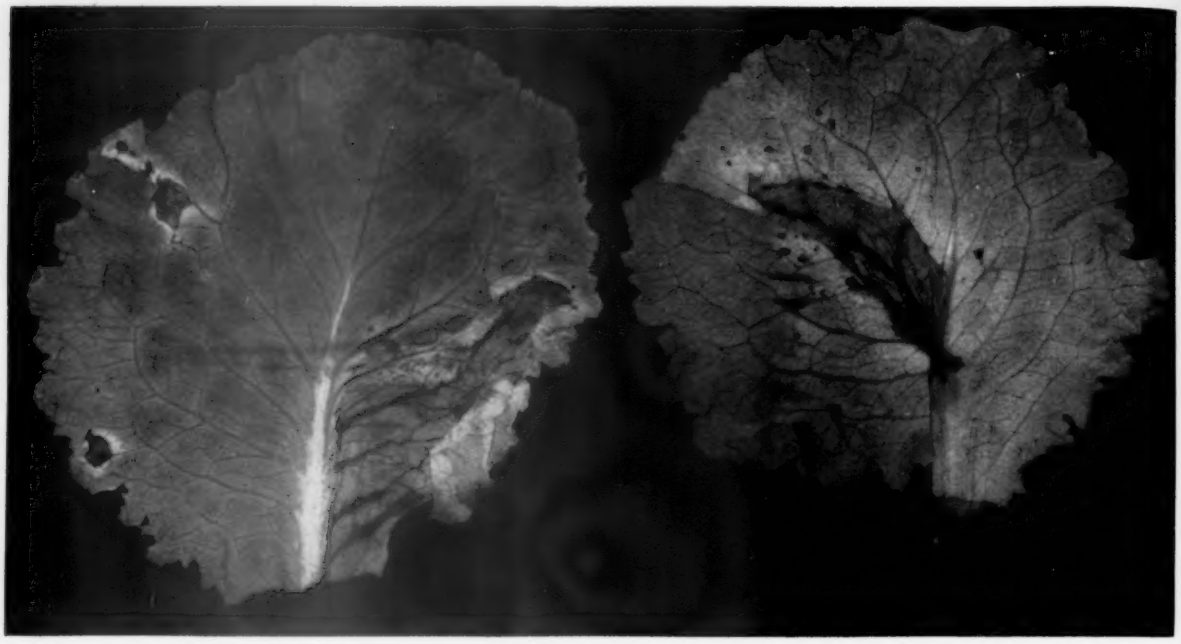

FIG. 1. Artificial inoculation of leaves of head lettuce. Right) *Xanthomonas lactucae-scariolae*. Left) *X. vitians*.

ever, be done before definite conclusions can be reached.

Pelargonium and calla plants showed no evidence of infection when inoculated with *P. cichorii* or *P. marginalis*.

Other green fluorescent plant pathogens were tested for ability to produce a potato rot. One isolate of the following was used in the experiments except that 10 isolates of *Pseudomonas syringae* van Hall were used. These pathogens were *P. angulata* (Fromme & Murray) Holland, *P. apii* Jagger, *P. glycinea* Coerper, *P. maculicola* (McCulloch) Stevens, *P. sesami* Malkoff, *P. tabaci* (Wolf & Foster) Stevens, *P. tomato* (Okabe) Alstatt, *P. viridilivida* (Brown) Holland, *P. viridiflava* (Burkholder) Clara, and *P. viburni* (Thornberry & Anderson) Stapp. Of these bacteria, only *P. viridiflava* produced a rot of potato, and here again is brought forth a similarity between *P. cichorii* and *P. viridiflava*.

Since *Pseudomonas cichorii* and *P. marginalis* have a varied host range, the bacteria probably have a better chance of over-wintering and being disseminated than if they were pathogenic to lettuce only. Furthermore, since it has been shown in these investigations that these 2 species of *Pseudomonas* have the ability to produce soft rots in vegetables the questions arise: how extensive is their distribution and how often have they been mistaken for *Erwinia carotovora* and other *Erwinia* species?

CULTURAL STUDIES.—Cultural studies were made of the 17 isolates obtained from rotting head lettuce in N. Y. State; together with cultures of *Pseudomonas endiviae* (*P. cichorii*) isolated by W. Kotte from endive in Germany in 1929, *P. viridilivida* (A.T.C.C. 512), *P. marginalis* received from B. A. Friedman isolated from witloof chicory, *P. syringae* isolated by the writer from lilacs in 1946, and *X. lactucae-scariolae* received from H. W. Anderson 1949.

The methods used in the culture studies were the standard methods (5). One biochemical reaction that should be especially mentioned is that of the reduction of nitrates to nitrites. In the literature there has been some confusion as to whether or not certain species of *Pseudomonas* do or do not reduce nitrates. Burkholder and Starr (3) lists 14 species as reducing nitrates to nitrites where the original description reported them negative. In the present investigations only 2 of the 6 isolates of *P. marginalis* showed positive nitrate reduction in broth. However, 5 isolates gave a positive nitrite test when grown in a synthetic nitrate solution. Thus only 1 isolate appeared to be negative. One isolate only of *P. cichorii* gave a positive test in broth, but all 9 isolates gave a positive test in a synthetic solution. In these studies it has been shown that both pathogens have the ability to reduce nitrates to nitrites under certain conditions. Therefore, a separation of the 2 species on this basis is impossible.

PSEUDOMONAS CICHORII (SWINGLE) STAPP.—Nine isolates from diseased lettuce, together with the isolate of *Pseudomonas endiviae* from Germany gave similar reactions in culture and were considered to be *P. cichorii*. Previously listed as synonyms of this species (1) are *P. endiviae* and *Bacterium formosanum*.

*Morphology*.—The pathogen is a non-spore-forming slender rod, occurring singly or in pairs. In a 24-hour-old culture on beef-extract-peptone agar the average measurements are 2.2 by 0.8$\mu$. It is gram-negative and motile with 1 to several polar flagella.

*Cultural characteristics.* — On beef-extract-peptone agar slants, growth is light with undulating margins, smooth, butyrous, and colorless to white. The pathogen is viable on this medium 6 months or more at 12°C. without being transferred. Nutrient broth becomes turbid in 24 hours with no pellicle. Litmus milk is not cleared, but a slight reduction of litmus takes place at the bottom of the tube. Litmus becomes blue. Green fluorescent pigment develops in Uschinsky's and in Clara's solution. Growth in Fermi's solution is slight. Krumwiede's triple sugar agar becomes red. Endo's medium remains pink but the bacterial growth becomes a deeper pink than the medium.

*Physiological characteristics.*—Gelatin is not liquefied. Nitrites are produced from nitrates, but $H_2S$ is not formed. No action on fat is detectable on Starr's spirit blue agar. Acid but no gas is formed in a synthetic medium (Koser solution minus the citrate) plus the following: dextrose, galactose, levulose, arabinose, xylose, and mannitol. Slow growth develops in sodium citrate and in glycerol; sodium malonate and sodium tartrate become alkaline. No growth appears in lactose, salicin, sodium hippurate, ethyl alcohol and dulcitol. Reaction with sucrose is variable. Starch is not hydrolysed and only a few isolates liquefied a pectate medium. Growth occurs in 5 per cent NaCl but none in 7 per cent NaCl. Optimum temperature at 27°-30°C. Growth occurs at 6°C. but only a trace at 37°C.

PSEUDOMONAS MARGINALIS (BROWN) STEVENS.—Five isolates from lettuce together with the isolate *P. marginalis* from Friedman were identical in culture. *P. intybi* (Swingle) Stapp has a rather inadequate description but is so similar to *P. marginalis* in pathogenicity and in cultural characteristics that it can only be considered a synonym of *P. marginalis*. A culture, however, was not available for this work.

*Morphology.*—The pathogen is a slender rod, sometimes slightly curved, occurring singly, in pairs or short chains. In a 24-hour-old culture on beef-extract-agar cells are variable in size but average 2.7 by 0.8$\mu$. They are gram-negative and are motile with 1 to several polar flagella.

*Cultural characteristics.*—On beef-extract-peptone agar slants, growth is light with undulating margins, smooth, butyrous, glistening to dull, colorless to white but after several weeks yellow. A slight pinkish color appears in the medium with certain isolates. The pathogen is viable on this medium 6 months or more at 12°C. without transferring. Broth is turbid in 24 hours, but no pellicle is formed. Litmus in litmus milk is reduced, and the milk rapidly clears; medium becomes greenish in hue. Green fluorescent pigment develops in Fermi's, Uschinsky's and in Clara's solutions. Krumwiede's triple sugar agar becomes red. Endo's agar remains pink but colonies are a deeper pink than the medium.

*Physiological characteristics.*—Gelatin is liquefied. Nitrites are produced from nitrates. Action on fat is detectable on Starr's spirit blue agar. $H_2S$ is not formed. Acid but no gas is produced in synthetic medium plus dextrose, galactose, levulose, arabinose, xylose, glycerol, and mannitol; and alkali in sodium malonate. No growth occurs in lactose, salicin, sodium tartrate, dulcitol, sodium hippurate, and ethyl alcohol. Action on sucrose is questionable. Starch is not hydrolysed and only 2 isolates liquefied a pectate medium. Growth occurs in 5 per cent NaCl. No growth or only a trace is found in 7 per cent. Optimum temperature is 27°–30°C. There is slow growth at 6°C. and a trace at 37°C.

XANTHOMONAS VITIANS (BROWN) DOWSON.—Three species of *Xanthomonas* have been described as pathogenic on *Lactuca spp.*, *Xanthomonas vitians* and *X. lactucae* on *L. sativa*, and *X. lactucae-scariolae* on *L. scariolae* (7). This last named species was reported not to infect *L. sativa* (15). An isolate of it, however, received from Dr. H. W. Anderson produced similar lesions on the common head lettuce as did the 3 isolates of *X. vitians*. Also the 3 New York isolates and *X. lactucae-scariolae* in culture were similar. In literature the differences between *X. vitians* and *X. lactucae* are so slight as to be no more than strain differences. *X. lactucae* and *X. lactucae-scariolae* therefore are considered synonyms of *X. vitians*, the first described species.

*Morphology.*—The pathogen is a non-spore-forming straight rod with rounded ends, occurring singly or in pairs. In a 24-hour-old culture on beef-extract-peptone agar, cells are approximately 2.1 by 1.0$\mu$. They are gram-negative and motile with a single polar flagellum.

*Cultural characteristics.*—On potato dextrose agar slants, growth is abundant, mucoid, yellow, and typical of all the species of *Xanthomonas*. Cultures remain viable without transferring 6 months or more on this agar at 12°C. but not on beef-extract-peptone agar. Broth becomes turbid in 24 hours but no pellicle is formed. In litmus milk a soft curd is formed in the bottom of the tube, milk is cleared, and litmus is reddish in color. Growth occurs in Fermi's solution but not in Clara's nor in Uschinsky's solution. Slight to no growth occurs on Endo's agar. Good growth occurs on Krumwiede's triple sugar agar and the medium turns a deep red.

*Physiological characteristics.*—Gelatin is liquefied. $H_2S$ is produced. Action on fat is detectable on Starr's spirit blue agar. Nitrates are not reduced to nitrites. Acid but no gas is produced in a synthetic medium plus dextrose, galactose, levulose, lactose, and sucrose. Slow growth occurs in sodium citrate. No growth appears in arabinose, glycerol, mannitol, dulcitol, salicin, and sodium hippurate and tartrate. Trace of growth occurs in malonate. Starch is not hydrolysed, or slightly so, but pectate medium is liquefied. No growth in 5 per cent NaCl. Growth is light at 6°C. but absent at 37°C.

An isolate of *Pseudomonas viridilivida* received many years ago from the American Type Culture Collection was included in the study. The characteristics of the isolate agree with a little variation from the original

TABLE 1.—*Comparison of cultural characteristics of 3 bacteria pathogenic on lettuce*

| Media or test | *P. cichorii* | *P. marginalis* | *X. vitians* |
|---|---|---|---|
| Colonies | White, butyrous | White to yellow, butyrous | Yellow, mucoid |
| Uschinsky's solution | Turbid, green | Turbid, green | No growth |
| Fermi's solution | Slight growth | Good growth | No growth |
| Gelatin | No liquefaction | Liquefaction | Liquefaction |
| Litmus milk | No clearing, alkaline, blue | Rapid clearing, greenish | Soft curd at bottom, reddish |
| Spirit blue agar | No reaction | Positive reaction | Positive reaction |
| Nitrates | Reduced | Reduced | Not reduced |
| $H_2S$ | Not produced | Not produced | Produced |
| Tartrates | Utilized | Not utilized by most isolates | Not utilized |
| 5% NaCl | Growth | Growth | No growth |

description. However, it can not be considered a synonym of either *P. cichorii* or of *P. marginalis*. It appears to be a distinct species. Nothing similar to it was found infecting lettuce in New York State. The cultural and physiological characteristics of the isolate of *Pseudomonas syringae* used in these experiments agreed with the descriptions of this pathogen given by other investigators. It was different, however, from the bacterial pathogens of lettuce reported above.

The main cultural differences noted in this study among the 3 bacterial pathogens of lettuce are set forth in Table 1.

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# AN EPIDEMIC OF PHYTOPHTHORA LEAF FALL OF HEVEA RUBBER TREES IN COSTA RICA[1]

J. B. Carpenter[2]

SUMMARY

A destructive epidemic of Phytophthora pod rot and leaf fall in a planting of 5- and 6-year-old hevea rubber trees in Costa Rica is described. The progressive development of defoliation was measured by making periodic counts of fallen leaves at 41 uniformly distributed 1-meter-square leaf collecting stations. The loss of branch wood, in linear meters, and the number of terminal shoots killed were determined for 22 trees at 12 representative stations.

From July 23 to August 25, 1953, when the epidemic was most severe, cumulative defoliation amounted to 615,500 leaves per hectare, representing 2565 kg. or 2.56 long tons of fresh leaves. The highest daily rate of leaf fall was 30,300 leaves or 126 kg. per day per hectare for a period of 6 days. Loss of branch wood amounted to more than 100 linear m. per tree in some cases, and was accompanied by a correspondingly high loss of terminal shoots, which bear the great bulk of the foliage. Thousands of valuable seeds were lost as a result of pod rot.

An unique opportunity to record from the start an epidemic of Phytophthora leaf fall of the hevea rubber tree occurred in Costa Rica in 1953. Numerical data were taken in order to present the development and effect of that epidemic in more concrete terms than those of simple observation, which has been the usual basis of the many references in the literature to similar occurrences.

*Phytophthora palmivora* Butler is one of the most aggressive parasites of the rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. and other species, and is present in some degree in all the principal rubber-growing areas of the world. All the aerial portions of the plant are susceptible to infection by this fungus. The symptoms and epidemiology of the disease in its several phases are given by Sharples (7), including pod rot, leaf fall, dieback, tapping panel decay, cankering of trunk and limbs, and collar rot Bugnicourt (2) and Darley and Silverborg (4) have reviewed the later work and report recent mycological investigations and studies on the development and control of panel decay.

Among the many reports on Phytophthora leaf fall in the Far Eastern plantations, apparently only that by Bertrand (1) has evaluated quantitatively the substantial loss of foliage. Bertrand reported from Ceylon that leaf fall was so severe in 1923 that on many estates the fallen leaves, spread out, would have covered the whole acreage. He estimated that for an estate of 600 acres the daily loss of elaborated plant food was about 10 tons. This would equal 33 lbs. per acre and, in contrast, the conservative data reported herein for Costa Rica indicate an average loss of more than twice that amount, or 69 lbs. per acre, for a 33-day period during the epidemic under consideration. These figures are only for leaves and ignore the loss of branches and seeds, dealt with later.

The Phytophthora disease first became generally severe on rubber in the Atlantic Zone of Costa Rica in 1950, where its great destructiveness and the control measures employed have been described by Langford et al (5) and Manis (6).[3]

The data in this paper were obtained at the U. S. D. A. Cooperative Rubber Plant Substation at Los Diamantes, near Guapiles, Costa Rica, during the period July to December 1953. The Los Diamantes Substation is situated on the gently sloping northern part of the Atlantic coastal plain. It has an elevation of approximately 135–270 m. above sea level and an annual rainfall of 442 cm. (10-year average). The epidemic occurred in an isolation seed garden of *H. brasiliensis* from the Department of Madre de Dios, Perú. This was the only disease-free planting of susceptible material in Costa Rica from which such information could be secured, as the disease had occurred already throughout the other plantings at Los Diamantes, in a 1000-hectare commercial rubber plantation at Cairo, and at the rubber research station in Turrialba.

METHODS.—Defoliation was measured at 41 leaf collecting stations, well distributed over the planting. There was 1 in every third row of trees along each of 4 parallel paths. The paths were 36 m. apart and the stations about 20 m. apart along the paths. Each station was a 1-meter-square area (Fig. 1) staked off under 1 or 2 trees. The squares, including surrounding margins of 0.5 m., were laid out and cleaned on July 23 and sprayed with diesel oil. The oil spray, repeated monthly, reduced maintenance costs.

Defoliation was recorded at stated intervals as the number of leaflets per station to avoid fractions (3), for the typical rubber leaf has 3 leaflets (Fig. 1). Any leaflet was counted that had more than half its area

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[3] The important rôle of E. P. Imle in the selection of Phytophthora-resistant clones discussed by these authors should be noted here, in recognition of his past and continuing contributions to this work.

FIG. 1. A typical 1-meter-square leaf collecting station with a few undisturbed, freshly fallen leaves showing the characteristic shedding of whole leaves that results from petiolar lesions caused by *Phytophthora palmivora* on the hevea rubber tree.

within the square. From these data the average rate of leaf fall, as whole leaves, was calculated for daily or longer periods and for both individual stations and larger areas. Shedding of whole leaves, due to petiolar infections, is a characteristic of Phytophthora leaf fall (Fig. 1). Drift of fallen leaves was found to be negligible because of the thick, slashed ground cover in the tree lines and the unslashed brush, about 2 m. high, between the lines.

Minimal values for the amount of foliage lost per unit area were calculated on the following bases: a) The numerical rate of leaf fall per sq. m. was determined from the averages for the 41 stations for the desired time interval and these converted into values for hectares or acres. b) The weight of the foliage lost was based on the average weight of 100 fresh, whole leaves, 417 gm., multiplied by the appropriate numerical rate of leaf fall. Leaf weight was determined from a representative sample of 800 whole leaves (total from 97 flushes) from the larger to medium-sized trees. c) The values in *a* and *b* were reduced by ⅓ to compensate for the land area not directly under the trees. It was estimated that more than ⅔ of the land was originally covered by the crowns before defoliation occurred.

To measure the loss of branch wood resulting from leaf fall and direct infection, the dead branches from 22 trees at 12 representative stations were knocked down and measured as linear meters of branch wood. The number of dead terminal shoots per tree was recorded also. These carry the great bulk of the foliage in trees of this age and older. Most of the branches were under 2 cm. in diameter; very few were over 4 cm. Dead branches were seen only infrequently in this planting prior to the outbreak of Phytophthora. All the damage noted herein may be attributed directly to that disease.

The diameters of the trees under which collections were made were measured at a height of 1 m. Severity of leaf fall is associated with heavy fruiting and this occurs mostly in the larger trees. However, trunk diameter does not always indicate the relative size of the crown nor fruitfulness, as there are great differences in growth habit among clones. In the area represented by stations 1–30, the trees were in mixed lines of cloned seedlings. Seed pods were common, though some clones were non-fruitful. Stations 31–41 were in a planting of younger, mixed seedling trees and only a few individual trees were fruitful.

RESULTS.—The main part of this isolation garden at the Los Diamantes Substation comprises 2.92 hectares and was planted in 1947 and 1948. Although contemporary plantings 2 kilometers or more from this garden, and separated from it by dense second-growth jungle, have suffered more or less severe damage from Phytophthora, the fungus had not been found in the isolation garden through July 3, 1953. When the garden was revisited on July 23, 2 small centers of severe infection were found in heavily fruiting trees. The fruit, or seed pod, has a firm, fleshy pericarp that is especially susceptible to infection by *P. palmivora* and the diseased fruits usually produce enormous crops of sporangia that serve as inoculum. Heavily fruiting trees are commonly the centers from which the disease spreads, both initially and in later years, following annual leaf change and flowering.

The course of the epidemic is outlined in Fig. 2, showing the almost explosive violence with which the disease may develop and spread, given favorable conditions. The primary focus of infection was probably in a group of large, heavily fruiting trees at station 26. These trees were already heavily defoliated by July 23. Past experience would indicate that the disease first appeared some 2 weeks earlier, about July 10.

On July 23 there was heavy infection and some leaf fall at stations 3 and 4. Light infections, with or without leaf fall, were seen also at stations 2, 5, 8, 9, 14, 15, 16, 17, 19, 25, 27, and 30.

Defoliation was recorded at all these stations 1 week later, July 30, and had begun at a few other stations. By August 6 the disease had developed greatly and defoliation was then heavy at stations 3, 4, 8, 9, 15, 16, 17, 19, 25, and 27. The trees at station 26 were almost bare. Some defoliation was recorded at nearly all other stations from 1 to 30. The smaller trees at stations 31–41 were still nearly disease-free. Within another week, on August 12, leaf fall was mostly heavy at stations 1–30 and had begun at stations 31–41.

During the following fortnight, when unfortunately no readings could be made, defoliation was general and heavy. By August 25, just 1 month after the disease was first found in this area, it had almost completely defoliated the trees and rotted the fruit on nearly all of the larger trees. Leaf fall was estimated to have removed more than 75 per cent of the foliage in the area including stations 1–30. Judging by the dried and browned condition of most of the leaves counted on August 25, probably heavy defoliation had begun to decline several days before. After that date, leaf fall was sparse and has been shown only to September 11, although it continued to at least October 7, when the final counts were made.

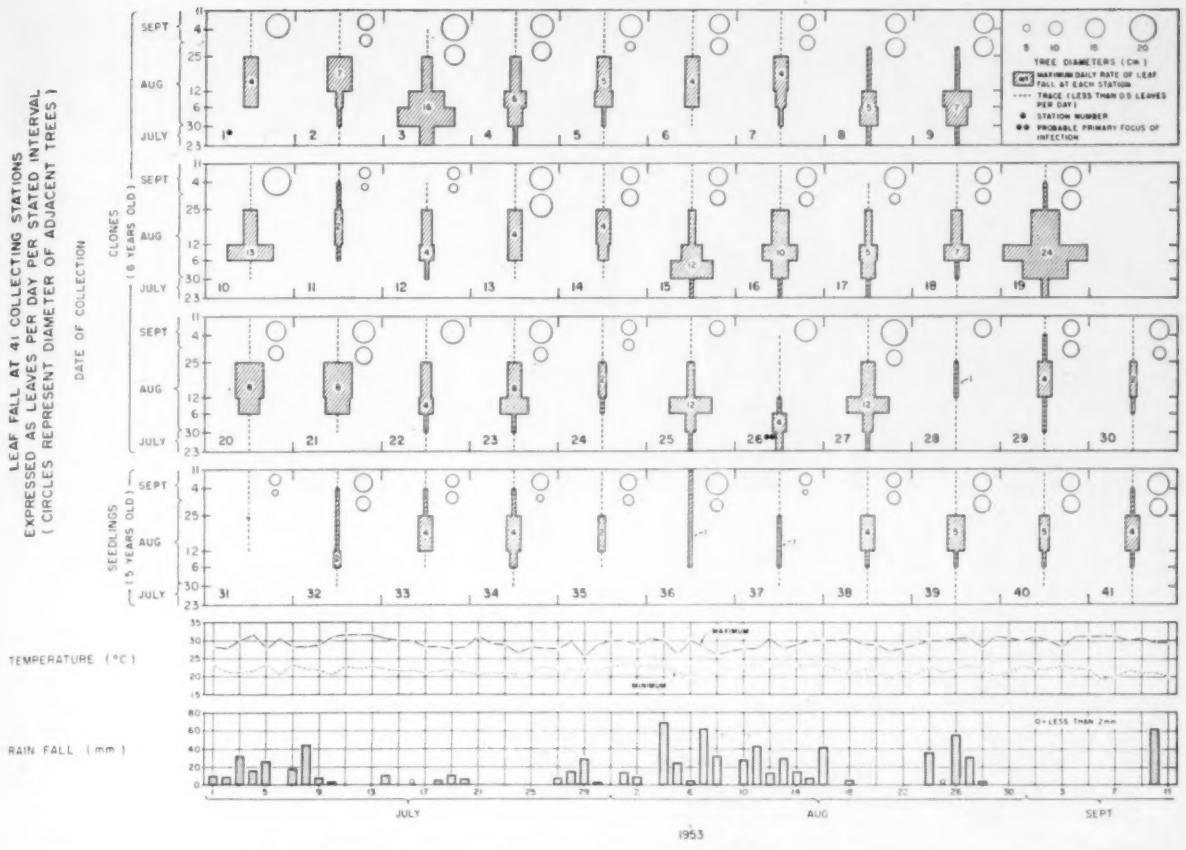
LEAF FALL AT 41 COLLECTING STATIONS
EXPRESSED AS LEAVES PER DAY PER STATED INTERVAL
( CIRCLES REPRESENT DIAMETER OF ADJACENT TREES )
DATE OF COLLECTION
CLONES ( 6 YEARS OLD )
SEEDLINGS ( 5 YEARS OLD )
SEPT
AUG
JULY
TREE DIAMETERS ( CM )
MAXIMUM DAILY RATE OF LEAF FALL AT EACH STATION
TRACE ( LESS THAN 0·5 LEAVES PER DAY )
STATION NUMBER
PROBABLE PRIMARY FOCUS OF INFECTION
TEMPERATURE ( °C )
MAXIMUM
MINIMUM
RAIN FALL ( mm )
0 = LESS THAN 2 mm
JULY
AUG
SEPT
1953

FIG. 2. The progressive development of Phytophthora leaf fall in a 2.92-hectare planting of 5- and 6-year-old hevea rubber trees of Madre de Dios, Perú, origin at Los Diamantes Substation, Guapiles, Costa Rica. Leaf-fall data were taken periodically at each of 41 1-meter-square leaf collecting stations. The results are given as the average number of fallen leaves per day per period at each station. Tree diameter, 1 m. above ground, is shown since severity of leaf fall is associated with substantial fruiting, which occurs mostly in the larger trees. Weather data indicate the prevailing meteorological conditions.

The remarkably heavy leaf fall in the trees at station 19 was probably due to the growth habit of that clone. It formed one of the larger crowns, composed of many fine terminal branches bearing heavy foliage, and had numerous seed pods. The damage from defoliation is reflected in the correspondingly large loss of short terminal shoots and branch wood (Table 1).

Partial refoliation began in late September, but the planting remained in poor condition through December. It probably will not improve until normal leaf change occurs in early 1954, but only a temporary respite from further damage by Phytophthora should be expected.

Pod rot and leaf fall are favored by prolonged periods of frequent rainfall, moderate temperatures, and high humidity. These conditions existed from July 1 to August 28 (Fig. 2). High humidity, although not recorded, prevailed during that period. There was less rainfall and lower humidity during the frequent bright days thereafter. Daytime temperatures were somewhat higher. The sharp decline in defoliation recorded after August 25 reflects the combined effects of previous defoliation, less favorable weather conditions, and greatly increased ventilation in the area comprising stations 1–30. The delayed and lighter infection found at stations 31–41 probably resulted from the general lack of fruiting trees and the drier weather that began in late August. Also, these stations are located on the eastern, upwind side of the planting, which may have retarded the spread of Phytophthora into that area.

The heaviest daily rate of leaf fall occurred during the period of August 6 to 12, amounting to an average of 4.54 leaves per day per sq. m., or 30,300 leaves per hectare per day, with a weight of not less than 126 kg. (12,300 leaves per acre, or 113 lb.).

Though these data on the damage caused by Phytophthora are mostly qualitative, they indicate the course of the disease. Some quantitative results have been calculated in order to emphasize the seriousness of the leaf-fall and branch-dieback phases of this disease.

Loss of foliage for the period July 23 to August 25 amounted to 92.3 leaves per sq. m., or 615,500 per hectare, with a weight of 2565 kg. or 2.56 long tons (249,000 leaves per acre, or 1.14 tons).

Striking numbers of dead branches were evident when refoliation began in late September. This has

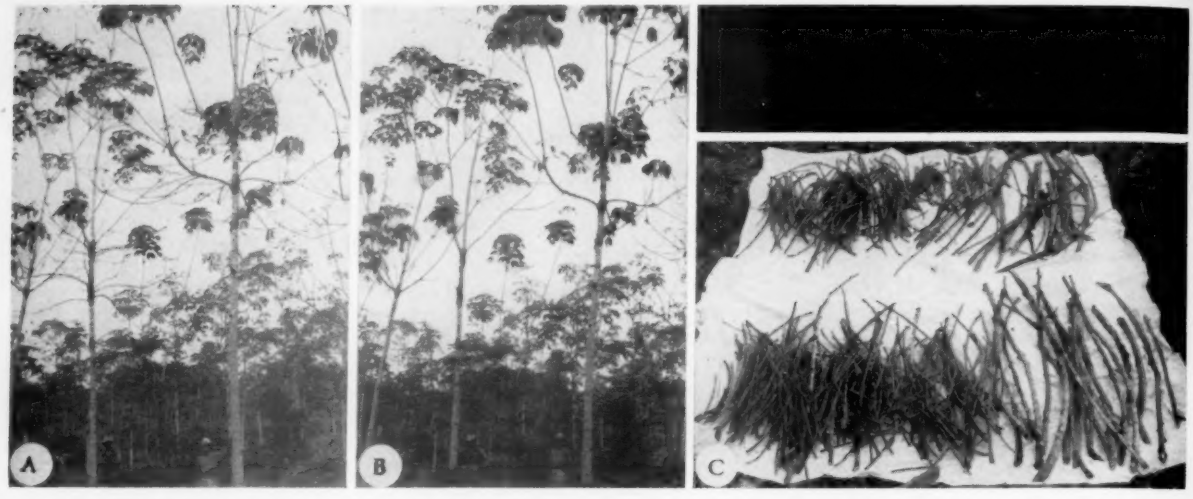


FIG. 3. Dieback of hevea rubber trees resulting from an epidemic of Phytophthora pod rot and leaf fall, showing trees at station 4 both before A) and after B) removal of dead branches. In C) the yields of dead branch wood from the larger tree on the right (top) and the smaller tree (bottom) represent 132 and 156 linear meters, respectively. Refoliation may be seen in the larger tree and some of those in the background.

been a characteristic aftermath of severe leaf fall, at least under Costa Rican conditions. Since most of the branches slough within a few months and decay rapidly, the importance of this loss sometimes is not recognized in judging the over-all effects of this disease.

A typical instance of branch dieback in larger trees is illustrated in Fig. 3, showing the same 2 trees both before and after removal of the dead branches and the quantity of dead wood from each tree.

Measurement of dead branch wood furnished another index of the destructiveness of the Phytophthora disease. The data were taken on November 19 and 20, when primary dieback[4] had run its course and the branches were dry enough to be knocked down (Table 1). The results indicate the relationship that might be anticipated among leaf fall, numbers of terminal shoots killed, and length of dead branch wood. The lack of smoothly proportionate increases in all categories is ascribed to clonal and individual tree differences and the location of the leaf collecting stations. Many of the more severely damaged trees were estimated to have lost well over 50 per cent of their total branch length.

TABLE 1.—*Comparison of number of terminal shoots killed, representative leaf fall, and amount of dead branch wood resulting from infection of hevea rubber trees by Phytophthora palmivora* [a]

| Collecting station | Diameter of trees | | Totals for each station: Length of dead branches | Terminal shoots killed | Leaves collected in 1-meter-squares July 23–August 25 |
|---|---|---|---|---|---|
| No. | Centimeters | | Linear meters | No. | No. |
| 35 | 8.6 | 12.5 | 18 | 32 | 30 |
| 34 | 12.8 | ..... | 41 | 66 | 62 |
| 39 | 13.1 | 13.8 | 41 | 84 | 71 |
| 22 | 17.3 | ..... | 45 | 62 | 72 |
| 12 | 6.7 | 9.9 | 71 | 114 | 69 |
| 17 | 7.7 | 16.3 | 73 | 102 | 85 |
| 18 | 11.8 | 13.1 | 87 | 186 | 92 |
| 13 | 17.3 | 17.6 | 132 | 194 | 76 |
| 16 | 11.2 | 15.4 | 152 | 216 | 165 |
| 27 | 12.2 | 20.2 | 217 | 277 | 149 |
| 4 | 14.4 | 17.6 | 288 | 290 | 112 |
| 19 | 14.7 | 15.0 | 318 | 658 | 355 |

[a] The collecting stations, trees concerned, and the meter squares on which leaf-fall data were taken are the same as those in Fig. 2.

Another category of appreciable loss was that of many thousands of valuable rubber seeds for establishing nurseries in late 1953. Ever since Phytophthora became severe in 1950, there have been no worthwhile rubber seed crops in Costa Rica. This has forced the importation of seed from other countries at heavy expense, both in actual cost of seed and transportation and in reduced germination as a result of delays in planting.

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[4] In contrast to this, a type of slow secondary dieback of the weakened, larger branches often occurs over a period of several months.

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# THE INFLUENCE OF DOUGLAS FIR SAWDUST AND CERTAIN FERTILIZER ELEMENTS ON THE INCIDENCE OF RED-STELE DISEASE OF STRAWBERRY[1]

Edward K. Vaughan, A. N. Roberts, and W. M. Mellenthin[2]

SUMMARY

Douglas-fir sawdust, used as a mulch, caused a significant increase in the incidence of red-stele of strawberry, caused by *Phytophthora fragariae* Hickman. Similar amounts of sawdust incorporated with the soil caused only a slight increase in incidence of the disease. The increased development of red-stele in mulched soils was apparently brought about by a decrease in soil temperature and an increase in the moisture content of the soil, and was not influenced by the utilization of nitrogen by bacteria in the decomposition of sawdust.

Excessive nitrogen fertilization also increased the incidence of red-stele infections. In 4 years of testing, applications in excess of 100 lb. actual nitrogen per acre per year resulted in increased percentages of diseased plants. Phosphorus and potash did not affect red-stele development.

The value of Douglas fir sawdust in the production of horticultural crops has been under investigation at the Oregon Agricultural Experiment Station for a number of years.[3,4,5] The primary objective of these experiments was to determine the value of sawdust as a mulch or oganic soil amendment in soil management for strawberry production, and the amount of supplemental nitrogen required in such a program. Whereas the effect of sawdust on fungus diseases was not included in the original plans, the appearance of the red-stele disease (*Phytophthora fragariae* Hickman) in the plots made a consideration of this disease imperative.

Methods and materials.—The experiments were conducted at the Lewis-Brown Horticultural Farm southeast of Corvallis on a Chehalis silty clay loam soil. For 2 years prior to planting the strawberries this soil had been planted to Sudan grass. In the summer of 1948 the Sudan grass was plowed under and the soil was prepared by discing. The experiment was designed as a randomized split plot with 4 replications. The primary objective was to determine the effects of sawdust incorporated in the soil, sawdust as a surface mulch at the same rate as used in the soil, and clean cultivation without the addition of sawdust. A secondary objective was to determine the effect of 7 rates of nitrogen in combination with a constant level of phosphorus and potassium, and the effect of nitrogen and phosphorus-potassium controls.

Douglas fir sawdust recently received from the mill and containing approximately 178 per cent by weight of water was applied at the rate of 166 tons per acre (approximately 60 tons dry weight) to 1 series of blocks in the fall of 1948 and rototilled into the upper 6–8 in. of soil. In the spring of 1949, all plots were planted with Oregon Experiment Station strawberry hybrid 2001. In June, when the plots were well established, a series of plots was mulched with approximately 4 in. of the same lot of sawdust. These mulched plots were not cultivated after the sawdust was applied. A third series received no special treatment. Each plot consisted of 1/166 acre and contained 5 rows of 10 plants each, set 18 in. apart in rows 42 in. apart. There were guard rows between plots and blocks.

All fertilizer treatments were applied as side dressings on both sides of the rows. The annual amount of phosphorus and potassium was applied in the spring. The nitrogen applications were split, one half being applied with the phosphorus and potassium 1 month after planting, the other half in early August or prior to the normal period of bud differentiation. For each rate of nitrogen applied with phosphorus and potassium there were plots that received a like amount in 1950 and plots that received only half that amount.

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Approved for publication as Technical Paper No. 839 by the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology and the Department of Horticulture.

[2] Plant Pathologist, Associate Horticulturist and Assistant Horticulturist, respectively, Oregon Agricultural Experiment Station.

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TABLE 1.—*Influence of nitrogen fertilizer on incidence of red-stele disease of strawberry selection OE 2001*

| Treatment No. | Treatment [a] 1949 | Treatment [a] 1950 | Percentage of plants affected by red-stele June 7, 1950 |
|---|---|---|---|
| 1 | 0— 0— 0 | 0— 0— 0 | 4.33 |
| 2 | 0—150—100 | 0—150—100 | 9.33 |
| 3 | 200— 0— 0 | 200— 0— 0 | 13.67 |
| 4 | 100—150—100 | 100—150—100 | 7.67 |
| 5 | 100—150—100 | 50—150—100 | 5.00 |
| 6 | 200—150—100 | 200—150—100 | 10.50 |
| 7 | 200—150—100 | 100—150—100 | 9.67 |
| 8 | 400—150—100 | 400—150—100 | 9.67 |
| 9 | 400—150—100 | 200—150—100 | 18.33 |
| | | L.S.D. .01 | 10.74 |

[a] Pounds per acre of nitrogen from ammonium sulphate, $P_2O_5$ from treble superphosphate, and $K_2O$ from muriate of potash. One-half of nitrogen in early spring, same time other materials were applied, balance in early August before bud differentiation.

The composition of the fertilizers and the rates of application of the various chemical elements are in Table 1.

In June 1950, the plants were examined, and the number in each plot that were obviously diseased was recorded. When the diagnosis was doubtful the plant was removed from the soil and the diagnosis was checked by sectioning the roots. Certainly somewhat larger percentages of the plants were affected than were recorded. Since yield records were to be taken, only the obviously infected plants could be recorded as diseased.

At the end of the harvest season of 1950, all plants were removed, the sawdust mulch was carefully removed from the soil and stored for re-use, and the plots were plowed and disced. In April 1951, the plots were again planted, using the Marshall strawberry variety. Fertilizer rates and methods of application were the same as in the previous experiment except for variations in the amount of nitrogen in some instances. Sawdust was returned to the mulched plots with sufficient new sawdust to provide a 4-in. mulch. Methods of recording data were the same as in the previous experiment.

Both fertilizer and sawdust had a marked effect on development of red-stele. The data indicated, however, that each acted independently of the other, and that there was little or no interaction between the 2.

INFLUENCE OF FERTILIZER.—When fresh sawdust is incorporated with the soil, increased amounts of supplemental nitrogen, and in some cases phosphorus, are needed for several years after application to compensate for the temporary loss of these elements by soil bacteria that decompose the organic matter. With the exception of treatments 1 and 3, the rate of nitrogen was the only fertilizer variable.

In the first experiment there were significantly greater numbers of infected plants in those plots that received a high rate of nitrogen. Whereas abundant nitrogen during the fall and winter months tended to increase the development of red-stele, an abundance of the same element during the period of rapid plant growth the following spring apparently made it possible for some infected plants to grow sufficiently to escape detection.

In the second experiment, after 4 years of fertilization, the influence of nitrogen on the development of red-stele was much more pronounced. Possibly this reflects the cumulative effect of more than 1 season of heavy nitrogen fertilization. In all plots which received more than 100 lbs. of nitrogen per acre per year, there was a significantly greater development of disease and those which received no nitrogen had the least red-stele (Table 2). Plots which received a total of 1600 lbs. of nitrogen during the 4 seasons (treatment 8) had by far the greatest incidence of disease. Those which received a total of 750 to 800 lb. of nitrogen (treatments 3, 6 and 9) had proportionate percentages of infected plants. Plots which received smaller total amounts of nitrogen during the 4-year period had less red-stele. The presence or absence of phosphorus and potassium appeared to have no influence on the development of the disease.

INFLUENCE OF SAWDUST.—In both experiments the influence of sawdust was much more striking and consistent than the effect of nitrogen fertilization. In the first experiment, the greatest development of disease was in the plots mulched with sawdust. Probably this resulted from a reduction in temperature and a marked increase in the moisture content of the soil under the mulch. The soil did not dry out rapidly at the end of the rainy season and remained at field capacity for several days after each irrigation. In the clean-cultivated plots where the disease caused the least damage, the soil dried rapidly after each irrigation and remained at field capacity for only a few hours at a time. The plots which had sawdust incorporated with the soil had only a slightly greater number of diseased plants than the clean-cultivated plots.

In the second experiment these differences were even

TABLE 2.—*Influence of nitrogen fertilizer on incidence of red-stele of Marshall strawberry*

| Treatment No. | Treatment [a] 1951 | Treatment [a] 1952 | Percentage of plants affected by red-stele June, 1952 |
|---|---|---|---|
| 1 | 0— 0— 0 | 0— 0— 0 | 7.83 |
| 2 | 0—150—100 | 0—150—100 | 7.83 |
| 3 | 200— 0— 0 | 200— 0— 0 | 28.33 |
| 4 | 100—150—100 | 100—150—100 | 13.00 |
| 5 | 25—150—100 | 12—150—100 | 15.33 |
| 6 | 200—150—100 | 200—150—100 | 30.17 |
| 7 | 50—150—100 | 25—150—100 | 11.50 |
| 8 | 400—150—100 | 400—150—100 | 42.83 |
| 9 | 100—150—100 | 50—150—100 | 24.67 |
| | | L.S.D. .01 | 13.88 |

[a] Pounds per acre of nitrogen from ammonium sulphate, $P_2O_5$ from treble superphosphate and $K_2O$ from muriate of potash. One-half of nitrogen in early spring, same time other materials were applied; balance in early August before bud differentiation.

TABLE 3.—*Influence of Douglas fir sawdust on the incidence of red-stele disease of strawberry*

| Sawdust Treatment | Percentage of plants affected by Red-stele 1950 | 1952 |
|---|---|---|
| Sawdust incorporated with soil | 8.67 | 16.61 |
| Mulched | 12.72 | 35.89 |
| Clean cultivated | 7.89 | 8.00 |
| L.S.D. .01 | not sig. | 10.82 |

more pronounced. The greater incidence of disease resulted partly from the fact that the Marshall variety is more susceptible to red-stele and partly from the fact that the mild, wet winters of 1951 and 1952 were much more favorable for the fungus than the unusually cold winters of 1949 and 1950. The greatest development of the disease occurred in those plots mulched with sawdust and the least in the clean-cultivated plots. There was not a significant increase in the number of diseased plants in the plots where sawdust was incorporated with the soil over that of the clean-cultivated plots.

CONCLUSIONS.—From the results of these experiments, 2 general conclusions can be reached. Applications of large amounts of nitrogen fertilizer increase the development of red-stele disease. Therefore, in soils where the fungus is known to be present and in poorly draind soils where the disease is most likely to develop, excessive nitrogen fertilization should be avoided. The use of sawdust as a mulch improves the growth and appearance of strawberry plants during the first season of growth. Its use for this purpose should be avoided in soils with red-stele, however, because red-stele causes much greater damage in mulched soils than in clean-cultivated soils.

The use of sawdust in or on the soil has been suggested to aid in restoring organic matter which is being depleted by continuous row crop cultivation. Whereas it is unquestionably of great value as a source of organic matter and may be used safely if mixed well with the soil, its use as a mulch on some soils may be extremely hazardous and should be avoided unless the grower is certain that the red-stele fungus is not present. The increased development of red-stele in soils mulched with sawdust is apparently not influenced by the utilization of nitrogen by bacteria in the decomposition of sawdust; rather, it appears to be brought about by a decrease in soil temperatures and an increase in the moisture content of the soil underneath the protective mulch.

OREGON AGRICULTURAL EXPERIMENT STATION
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# AN ANTIGENIC ANALYSIS OF SOME PLANT AND SOIL CORYNEBACTERIA [1]

Stanley A. Rosenthal [2] and Charles D. Cox

SUMMARY

Several species of plant and soil corynebacteria have been studied using agglutination and agglutinin-adsorption techniques.

*Corynebacterium poinsettiae*, *C. flaccumfaciens*, and *C. sepedonicum* appear to be serologically homogeneous species, as could be determined from the small number of strains available for this study. *C. poinsettiae* and *C. flaccumfaciens* could not be distinguished serologically, whereas *C. sepedonicum* and *C. tumescens* appear to be serologically distinct from each other and from the *C. poinsettiae—C. flaccumfaciens* group.

*C. poinsettiae*, *C. flaccumfaciens*, *C. sepedonicum*, and *C. tumescens* are antigenically unrelated to *C. michiganense* and *C. insidiosum*.

There is a noticeable lack of studies dealing with the antigenic structure of plant and soil members of the genus *Corynebacterium*. Since Conn and Dimmick[3], on the basis of morphological and physiological studies, have objected to the inclusion of plant and soil diphtheriods in the genus *Corynebacterium*, such studies may be of importance in determining the taxonomic position of these groups. Rosenthal and Cox[4] have reported that strains of *C. michiganense* and *C. insidiosum* share 1 or more antigens, and some strains of the 2 species appeared to be antigenically indistinguishable. The following experiments are a continuation of this latter study and include antigenic analyses of other plant species and 1 soil species (*C. tumescens*) now included in the genus *Corynebacterium*.[5]

[1] Accepted for publication June 12, 1954.

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[2] Present address: Bacteriology Section, Skin and Cancer Unit, University Hospital, 330 Second Avenue, New York, New York.

Atomic Energy Commission pre-doctoral fellow, 1951-1952.

[3] Conn, H. J., and Isabel Dimmick. 1947. Soil bacteria similar in morphology to Mycobacterium and Corynebacterium. Jour. Bact. 54: 291-303.

[4] Rosenthal, S. A., and C. D. Cox. 1953. The somatic antigens of Corynebacterium michiganense and Corynebacterium insidiosum. Jour. Bact. 65: 532-537.

[5] Breed, R. S., E. G. D. Murray, and A. P. Hitchens. 1948. Bergey's Manual of Determinative Bacteriology. Ed. 6. The Williams and Wilkins Co., Baltimore.

TABLE 1. *Antigenic inter-relationships among C. poinsettiae (CPO69), C. tumescens (CT47), and C. sepedonicum (CS850)*

| | Antisera | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CPO69 antiserum | | | | CS850 antiserum | | | CT47 antiserum | | |
| Antigens | Titer before adsorption | Titer after adsorption with antigen: CPO69 | CS850 | CT47 | Titer before adsorption | Titer after adsorption with antigen: CS850 | CPO69 | Titer before adsorption | Titer after adsorption with antigen: CT47 | CS850 |
| CPO69 | 640 | 0[a] | 320 | 320 | 80 | 0 | 0 | 0 | | |
| CS850 | 160 | 80 | 0 | 160 | 2560 | 0 | 2560 | 160 | 0 | 0 |
| CT47 | 160 | 80 | 80 | 0 | 0 | | | 10240 | 0 | 10240 |

[a] No agglutination at a serum dilution of 1–40 or higher.

EXPERIMENTAL METHODS.—The following cultures were used in these studies: *C. poinsettiae*, strains CPO82, CPO70 and CPO69 (A. T. C. C. #9682, 9070 and 9069); *C. flaccumfaciens*, strain CFL92 (A. T. C. C. #7392); *C. sepedonicum*, strain CS850 (A. T. C. C. #9850); and *C. tumescens*, strain CT47 (A. T. C. C. #6947). *C. poinsettiae*, strain CPO1; *C. flaccumfaciens*, strain CFL18, and *C. sepedonicum*, strain CS101, were from the collection of Dr. M. P. Starr at the University of California, Davis. Preparation of antigens and antisera, adsorption of antisera and tube agglutination tests were done as previously described.[4]

RESULTS AND DISCUSSION.—*Intra-species studies.*—Results obtained by cross-adsorption studies indicate that *C. poinsettiae*, *C. flaccumfaciens*, and *C. sepedonicum* each comprise a serologically homogeneous species as far as could be determined with the small number of strains available for study. Although antisera were not prepared against all strains of each species, the observation that heterologous strains within the species were agglutinated to titer and adsorbed all agglutinins for the homologous antigen supports the conclusion that these species are serologically homogeneous.

*Inter-species studies.*—Preliminary titrations indicated that serological cross-reactions occurred among these species, as well as with a single strain of *C. tumescens* (CT47). Where such cross-reactions occurred, they were investigated in greater detail, and are reported below.

a) *C. poinsettiae and C. flaccumfaciens.*—Since no serological differences could be detected among strains of *C. poinsettiae* and *C. flaccumfaciens*, strains CPO69 and CFL18 were chosen to represent these species in inter-species studies.

TABLE 2. *Antigenic inter-relationships among C. flaccumfaciens (CFL18), C. sepedonicum (CS850) and C. tumescens (CT47)*

| | CFL18 antiserum | | |
|---|---|---|---|
| Antigens | Titer before adsorption | Titer after adsorption with antigen: CFL18 | CS850 |
| CFL18 | 604 | 0[a] | 640 |
| CS850 | 80 | 40 | 0 |
| CT47 | 80 | 80 | 80 |

[a] No agglutination at a serum dilution of 1–20 or higher.

The results of titrations using adsorbed antisera against strains CPO69 and CFL18 indicated that *C. poinsettiae* and *C. flaccumfaciens* could not be distinguished serologically. Both antigens agglutinated to the homologus titer of each antiserum, and adsorption of CPO69 and CFL18 antiserum with either antigen removed all agglutinins against both antigens.

b) *C. poinsettiae, C. sepedonicum, and C. tumescens.*—The results of cross-adsorptions involving these species are recorded in Table 1. These data strongly suggest that only a minor antigenic relationship exists between *C. poinsettiae* and *C. sepedonicum*, and that no serological relationship exists between *C. poinsettiae* and *C. tumescens*. It may also be seen that *C. sepedonicum* and *C. tumescens* are antigenically unrelated.

c) *C. flaccumfaciens, C. sepedonicum, and C. tumescens.*—When CFL18 antiserum was adsorbed with its homologous antigen, the agglutinin titers to antigens CS850 and CT47 were not reduced (Table 2), indicating that such agglutinins to the heterologous antigens were not induced by immunization with antigen CFL18. These agglutinins probably represent naturally occurring antibodies to strains CS850 and CT47. This conclusion is substantiated by the observation that adsorption of CFL18 antiserum with antigens CS850 and CT47 did not reduce the titer of the serum to the homologous antigen. These results indicated that strain CFL18 does not share any antigenic components with strains CS850 or CT47.

Reciprocal agglutination studies using *C. michiganense* and *C. insidiosum* antisera and antigens on the 1 hand, and antisera and antigens of the species used in the present study on the other, were either negative or of a minor nature, indicating that *C. poinsettiae*, *C. flaccumfaciens*, *C. sepedonicum*, and *C. tumescens* did not contain the antigens A, B, or C[4]; nor did any of these latter species contain a demonstrable heat-labile, agglutination inhibiting substance.[4]

More detailed serological studies of plant and soil corynebacteria and their relationships to animal strains of this group may be of value in clarifying the taxonomic position of this group of microorganisms.

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*Chemical Measurements of Methyl Bromide Concentration in Relation to Kill of Fungi and Nematodes in Nursery Soil.* DONALD E. MUNNECKE AND DAVID L. LINDGREN. Methyl bromide gas is used extensively for control of weeds, nematodes, insects, and fungus pathogens in nursery soils. One hundred to 500 flats of soil are frequently fumigated in closely stacked piles which are enclosed beneath a gas-proof cover. The question is frequently raised as to whether the gas penetrates through such large stacks and is equally effective throughout the stack. This paper correlates the actual concentration of methyl bromide in the atmosphere beneath the cover and its effect on 6 species of fungi and the root knot nematode.

Two fumigation trials were made in a commercial nursery. Two hundred twenty-five flats of soil containing approximately 18 per cent peat moss were stacked in piles approximately 4.5 × 8 ft. on the sides and 5 ft. high. The test fungi and nematodes were enclosed in plastic screen bags, tagged and buried in flats in each of the 4 corners and the center at the bottom, middle, and top layers of flats. The technic of assessing the effect of the gas on the organisms has been described previously.[1] Plastic tubing was fastened to the flats in the center of the stack at the bottom, middle, and top layers so that a sample of the atmosphere at each location could be taken after the cover was in place. The flats were placed on one gas-proof polyethylene tarpaulin and covered with another, which was sealed by piling soil around the edges. Methyl bromide was injected at dosages of 4 lb. per 100 cu. ft. of space beneath the cover. The gas was metered out of a large cylinder into a calibrated glass cylinder, and then released beneath the cover at the top of the stack. The methyl bromide was vaporized by passing it through a copper coil immersed in hot water.

The temperature of the soil was 67–70°F. for the 2 tests made. The moisture content of the soil was not determined, but it was in correct condition for immediate seeding as is routine with nurserymen in this area. The treatment period was 24 hours. The concentration of methyl bromide in the atmosphere at the 3 positions was determined by the method of Stenger, et al.[2], with some modification. Samples of the atmosphere were taken at various periods from 15 minutes after the gas was injected until 24 hours later.

The concentration of methyl bromide gas at each position in the stack is shown in Fig. 1 for one test. The curve of the other test was essentially the same as that in Fig. 1. There was no appreciable difference

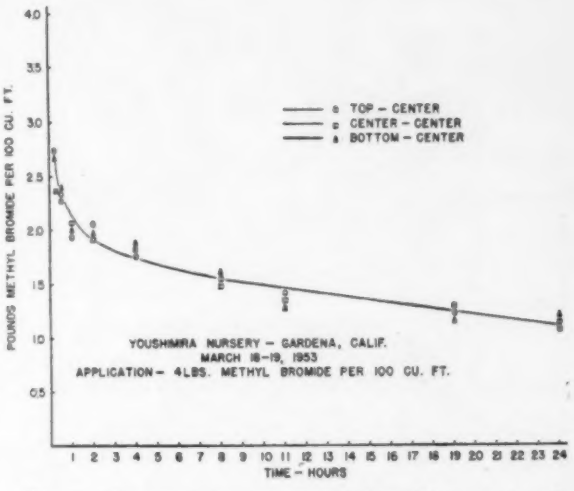
POUNDS METHYL BROMIDE PER 100 CU. FT.
TOP – CENTER
CENTER – CENTER
BOTTOM – CENTER
YOUSHIMRA NURSERY – GARDENA, CALIF.
MARCH 18–19, 1953
APPLICATION – 4 LBS. METHYL BROMIDE PER 100 CU. FT.
TIME – HOURS

FIG. 1. Concentration of methyl bromide gas in the atmosphere at 3 positions in a stack of 225 nursery flats filled with a mixed nursery soil at various intervals after treatment with 4 lb. per 100 cu. ft. of space.

in concentration of the gas at the 3 positions in the stack. Apparently a portion of the gas is sorbed by the soil very rapidly, since the chemical at the full 4 lb. per 100 cu. ft. rate was not recovered 15 minutes after injection and the concentration of the gas was found to have dropped very sharply to approximately 1½ lb. per 100 cu. ft. within the first few hours. After 3–4 hours the concentration curve leveled out and gradually decreased for the remaining 15–20 hours. Probably a large portion of this loss was due to leakage from around the edges of the cover and it was determined by use of a halide lamp that gas was escaping. This leakage is difficult to prevent since 4 lb. of methyl bromide occupy approximately 15 cu. ft., which creates considerable pressure until equilibrium is reached.

The effect on fungi and nematodes for both tests is shown in Table 1. These results are similar to those previously reported[1] and show that *Verticillium albo-atrum* is able to survive high concentrations of methyl bromide, whereas the other test organisms were killed. The survival of Verticillium inoculum was random throughout the stack and was not correlated to any specific position. Approximately 25 per cent of the inoculum pieces containing microsclerotia survived, whereas Verticillium in 80 per cent of the infested soil samples was unaffected by the treatment.

These tests show that methyl bromide gas rapidly penetrates a stack of flats and diffuses uniformly in the atmosphere within an hour after injection and does not stratify during the 24-hour treatment period. A major portion of the gas is sorbed by the soil in the first few

[1] Munnecke, D. E., and J. Ferguson. 1953. Methyl bromide for nursery soil fumigation. Phytopathology 43: 375-377.
[2] Stenger, V. A., S. A. Shrader, and A. W. Beshgetoor. 1939. Analytical methods for methyl bromide. Ind. Eng. Chem., Anal. Ed. 11: 121-124.

TABLE 1.—*Survival of fungi and nematodes in soil in flats treated with gaseous methyl bromide at the rate of 4 lb. per 100 cu. ft. of space for 24 hours*

| Organism tested | Type of inoculum | Percentage Survival Test 1 | Test 2 |
|---|---|---|---|
| *Sclerotinia sclerotiorum* | sclerotia | 0 | 0 |
| *Sclerotium delphinii* | sclerotia | 0 | 0 |
| *Pythium* sp. | mycelial mats | 0 | .... |
| *Rhizoctonia solani* | mycelial mats, sclerotia | 0 | 0 |
| *Fusarium oxysporum* f. *gladioli* | mycelial mats | 0 | 0 |
| | infected gladiolus cormels | .... | 0 |
| *Verticillium albo-atrum* | microsclerotia on sterile tomato stems, | 19 | 25 |
| | infested soil | .... | 80 |
| *Meloidogyne* sp. | root galls | 0 | .... |
| | infested soil | .... | 0 |

hours, after which a concentration in the air of about 1½ lb. per 100 cu. ft. is maintained. Except for *V. albo-atrum*, which frequently survived, pathogenic fungi and nematodes were killed equally well at all positions in the stack. The surviving inoculum was also found distributed at random through the stack.—Department of Plant Pathology, University of California, Los Angeles 24 and Department of Entomology, University of California, Riverside.

*Brick Red Stain of Sitka Spruce and Other Wood Substrata.* ROSS W. DAVIDSON AND FRANCES LOMBARD.[1] There are many fungi that stain or discolor wood. The most common and most pronounced are those causing the so-called blue stain of unseasoned sapwood. There are many others which cause superficial molding or only slight discolorations, most of which have not been thoroughly studied. One of these was recently described by Wells[2] as occurring on several types of wood and especially on white pine lumber in Canada.

This same fungus was isolated from heartwood of a Sitka spruce log at Astoria, Oregon, June 2, 1944, and from heartwood of the same host near Lake Quinalt, Washington, July 1, 1944. It could not be determined whether the fungus had been present in the trees before they were felled. However, it seemed to be present mainly in compression failures, possibly caused by shock in felling. There was a light pink discoloration associated but no surface growth of the fungus.

Later collections of it were made by E. Wright on Douglas-fir lumber in Oregon, April 8, 1946. In 1950 several samples of the surface-stained northern white pine lumber (Fig. 1, A) were sent to Beltsville, Maryland, from the Braeside, Ontario, location mentioned by Wells. The fungus was isolated from the white pine and proved to be the same as those from Oregon and Washington. Later it was observed growing on red oak heartwood (Fig. 1, B) which had been held in a moist chamber at the Beltsville, Maryland Laboratory.

In 1944 Hansbrough and Englerth[3] described some discolorations in aircraft lumber. As a supplement to this descriptive release they prepared sets of wood samples showing the common types of defects and discolorations of Sitka spruce lumber. One of these which was labeled "Brick red stain" contained some surface growth of *Ascocybe grovesii* Wells. The stain is undoubtedly caused either directly or indirectly by this fungus. Therefore, it seems appropriate to assign this name to the stain or discoloration caused by the fungus. "Brown stain" has already been used by Dr. Fritz[4] for stain in pine caused by the fungus *Cytospora* sp. or *C. pini* Desm. as reported by Zabel.[5] Zabel also calls attention to "pink to red stains" caused by *Fomes pini*.

The "Brick red stain" of Sitka spruce was partly superficial and was stated to be usually removed by the planer. The stain on white pine from Braeside seemed to be mostly due to a superficial growth. This was not the case in the 2 isolations made from logs of Sitka spruce in 1944. There the fungus had penetrated deeply into the heartwood but mainly following the very inconspicuous compression failures, apparently causing a light pink internal stain. The surface stain is due to some extent to the color of the red brown ascophore stalks. The mycelium is hyaline and would seem to cause little stain. The actual color caused by the fungus is quite variable ranging from pink to cinnamon brown and depending mainly on intensity of fungus growth on or in the wood.

*Ascocybe grovesii* is a very unusual fungus and when first studied could not be identified or placed in any of the known genera. The present writers had concluded that it should be described as a new genus and new species and placed near *Endomyces* and the paper by Wells solves the problem of a name for the brick red stain organism. It is a very interesting species which should be excellent for classroom studies in mycology. Cultures (Fig. 1, C) which were isolated

[1] Forest Pathologists, Forest Insect and Disease Laboratory, Fort Collins, Colorado and Forest Disease Research Laboratory, Beltsville, Maryland, respectively, U. S. Forest Service.

[2] Wells, Doreen E. 1954. Ascocybe, a New Genus of Lower Ascomycetes. Mycologia 46: 37-51.

[3] Hansbrough, J. R., and G. Englerth. 1944. The significance of the discolorations in aircraft lumber: Sitka spruce. Forest Pathology special release No. 21.

[4] Fritz, Clara W. 1952. Brown stain in pine sapwood caused by Cytospora sp. Can. Jour. Bot. 30: 349-359. [3 plates]

[5] Zabel, R. A. 1953. Lumber Stains and Their Control in Northern White Pine. Jour. of Forest Products Res. Soc. 3(3): 36-38.

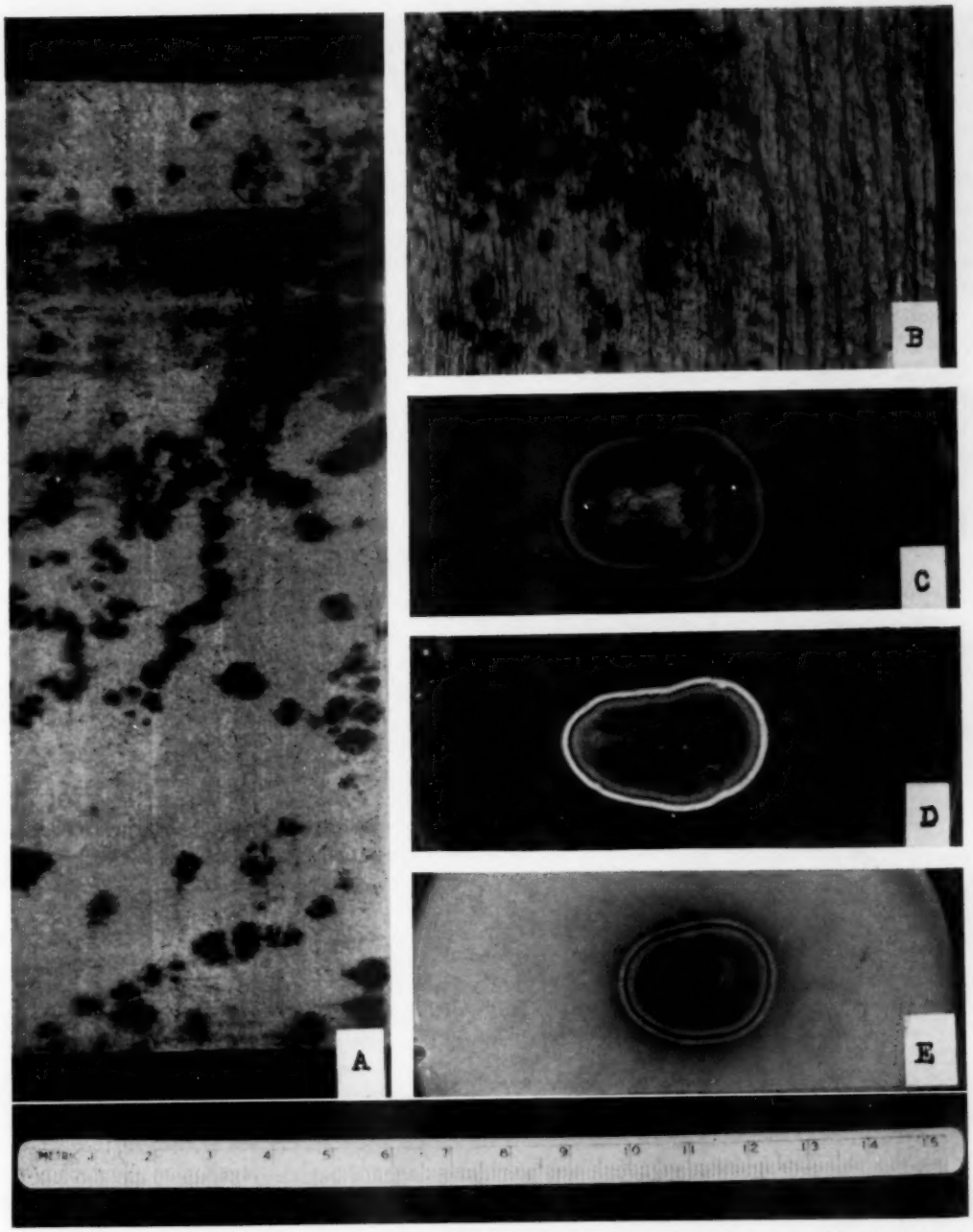


FIG. 1. *Ascocybe grovesii* Wells (cause of brick red stain on wood). A) Spots and streaks of brick red stain on eastern white pine; B) Spots of brick red stain on oak wood; C) Culture 11 days old on malt agar; D and E) Same age cultures on malt agar medium with 0.5 per cent gallic acid and 0.5 per cent tannic acid, respectively, added.

about 10 years ago appear to be almost exactly the same as when first obtained. In this respect it differs from many of the blue staining fungi and most Ascomycetes and fungi imperfecti which change radically when held in culture over long periods without special treatment in making transfers.

Growth rate was very little affected by the addition of gallic or tannic acid in a malt agar medium (Fig. 1, D and E). Growth on heartwood of such unrelated woods as oak and Sitka spruce also indicates that the fungus is not greatly affected by substratum. The production of sticky spore masses suggests that it may be insect associated, or disseminated by them.—Forest Insect and Disease Laboratory, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado.

*A Yellow Spot Virus in Potato Varieties in Aroostook County, Maine.* Reiner Bonde and Donald Merriam.[1] What appears to be an undescribed potato virus disease for Maine has been found in potato fields in Aroostook County. The disease was first noted in 1949 in several tuber units of an experimental Katahdin potato seed plot. Further observations have shown it is present in some farmers' seed stocks as well. To date it has been found mostly in the Katahdin variety. The disease, however, also was found in a few plants of the Sebago and Green Mountain varieties and recently was observed in several unnamed potato seedlings.

The disease is inconspicuous, easily overlooked in the field, and does not appear greatly to affect the growth or size of the plant. It apparently has escaped notice in Maine by the certified seed potato inspectors and others interested in growing healthy seed potatoes.

The symptoms consist of a few bright yellow spots mostly on the lower leaves. The number of spots varies from 1 to 5 or 6 per leaflet on a few of the leaves. The spots generally are nearly circular in shape and the margins definitely defined (Fig. 1). The spots may vary in size, but in most cases are rather small being about 1/8 in. or less in diameter. The symptoms resemble those produced by the apple mealy bug *Phenacoccus aceris* (Sign.) when feeding on the potato.[2]

[1] Plant Pathologist and Field Assistant, respectively, Maine Agricultural Experiment Station.

Fig. 1. Symptoms of yellow spot virus in lower leaves of Katahdin potato plant.

Table 1.—*Comparison of yields for katahdin seed potatoes with and without yellow spot virus*

| Seed stock | Yield bushels per acre[a] |
|---|---|
| Healthy | 600.7 |
| Yellow spot | 500.6 |
| Difference | 100.1 |

[a] L.S.D. at 1% level = 23.64 bushels.
Average of 10 replicated 10-hill plots for each seed stock.

The foliage symptoms also resemble those of tuber blotch incited by Solanum virus 8 (Clinch, Laughname and Murphy).[3] However, with yellow spot in Maine, we have not noted the necrosis such as described for Solanum virus 8.

The fact that yellow spot was observed in a number of unnamed seedling potato varieties that were heavily infected with aphids led the writers to suspect that the disease was transmitted by this insect. However, the disease was not transmitted by the peach aphid *Myzus persicae* Sulz. in controlled experiments, carried on for 2 successive years, in which about 100 young potato plants were each inoculated by placing 8 to 10 viruliferous aphids on the uppermost leaves. The leafroll virus, in contrast, was transmitted in nearly 100 per cent of the plants inoculated with the same insect. Also, symptoms of yellow spot were not produced by sap inoculations, although an interveinal type of mosaic appeared in a few inoculated Green Mountain potato plants. It is not known whether this symptom was caused by the yellow spot virus. The symptoms of yellow spot were, however, transmitted to 22 out of 100 Katahdin plants by the inarch graft method.

Although the disease does not materially affect the size and growth of Katahdin plants it caused a reduction in the yield rate. Table 1 summarizes the yield for a healthy Katahdin seed stock and one with yellow spot. The diseased seed stock yielded 100.1 bu. less per acre than that which was healthy.

Experiments have shown that the disease can be eliminated by planting tuber unit seed plots and by the careful selection and propagation of healthy tuber units. Observations to date indicate that the disease is not so readily disseminated in the field as are leafroll and some of the other virus diseases.—University of Maine, Orono, Maine.

[2] Simpson, G. W., and W. A. Shands. 1949. An unusual infestation of mealy bugs on potatoes. Maine Agr. Exp. Sta. Bul. 483, p. 45.

[3] Smith, Kenneth M. 1937. Textbook of Plant Virus Diseases. London.

*Some Factors Affecting Seed Transmission of Safflower Rust.* O. H. CALVERT AND C. A. THOMAS. *Puccinia carthami* Cda. is a seed-borne, autoecious, brachyform type rust of safflower, *Carthamus tinctorius* L.[1, 2] In addition to an undetermined loss from leaf pustules, the disease causes reductions in stands and is a menace to safflower production. Factors affecting seed transmission are of interest in developing control methods.

Experiments were designed to determine whether the rust could be transmitted by both urediospores and teliospores. Variety N8 safflower seed, heavily infested by dusting urediospores on the seed, were planted in pots of steamed soil in a greenhouse (temperature 20–24°C.). No rusted seedlings were produced from seed infested with urediospores which were known to be viable from laboratory germination tests and greenhouse inoculation of safflower leaves. In comparable tests an average of 5 per cent rusted seedlings were produced from teliospore infested seed. In other tests N8 seed, divided into lots of 48 seed each, were dipped into fresh undiluted egg albumin and then heavily coated with either urediospores or teliospores while still sticky. The seed were planted in flats and grown in a cool (5–15°C.) greenhouse. Two weeks after emergence the seedlings from urediospore covered seed showed no rust symptoms; however, 90 per cent of those grown from seed covered with teliospores were rusted and most seedlings severely so. One month later the plants grown from seeds coated with urediospores were still not infected. The infected seedlings in both experiments first exhibited spermogonia (pycnia) along the hypocotyl and on the cotyledons, which indicated that infection was accomplished from sporidia (basidiospores). It appears that the sporidia germinated and penetrated directly the seedling tissues underground during the seed germination process and before plant emergence.

Temperature appeared to be one of the more critical factors affecting seed transmission. Prasada and Chothia[2] tested teliospore germination at several temperatures and found 12–18°C. to be best with some germination occurring at 20–22°C. and none at 7–8° or at 26°C. and above. In preliminary experiments in which N8 safflower seed were infested with teliospores, it was noted that the percentage of infected seedlings from infested seed increased as the air temperature was lowered. A precise measure of the effect of temperature upon seed transmission was attempted in constant temperature chambers. Seeds of N8 were infested with freshly harvested teliospores and planted in steamed soil in 1-gal. crocks. Four crocks of 40 seeds each were placed at 5, 10, 15, and 20°C. After 1 week all the temperatures were raised to 22°C. for 1 month. In 2 tests an average of 96.1 per cent of the emerged seedlings were rusted at 5°C., 76.2 per cent at 10°C., 67.3 per cent at 15°C., and 29.3 per cent at 20°C.

Claassen[3] in 1950 noted that the varieties N3, N6, N10, and N852 were less susceptible to leaf pustules than N8, N9, N55, and Indian varieties. He also rated 4 lines, N976, 977, 2555, and 2591, as having either immunity or high resistance. The variety Western Oilseeds No. 14 is considered to be highly resistant. Seeds of varieties N8, N6, N10, and W.O. 14 were infested with teliospores, incubated for 7 days at 5°C. in moist, steamed soil, and grown at 22°C. for 1 month. In 6 tests, each consisting of 2 crocks of 40 seed of each variety, the following percentages of rusted plants were noted: N8–51.2, N6–32.5, N10–21.6, and W.O. 14–zero.

Prasada and Chothia[2] observed that 2 types of teliospores, morphologically similar, are formed in *P. carthami*: one with a limited period of viability and with ability to germinate soon after formation, and the other requiring a resting period. Teliospores were harvested from field plants at Mesa, Arizona, on June 23, 1953. They were sent to Beltsville, Maryland, and stored in a refrigerator at 2°C. During July and August, in tests similar to those described above, over 90 per cent infected plants were produced from N8 seed infested with those spores. In September and October the number of infected plants dropped to less than 1 per cent. In November, however, an average of 38 per cent infected plants were produced in 3 tests. Spores removed from the refrigerator in October and held at 25°C. for 4 weeks produced an average of 81.1 per cent infected plants in 2 tests.

Seed of the variety N8 harvested from rusted plants in a field in Nebraska in the fall of 1950 produced 20 per cent infected seedlings in tests made within 2 months after harvest. In the spring of 1952 this same seed lot, which had been stored in an unheated building at Lubbock, Texas, was retested. One per cent of the plants were rusted, indicating that some spores were still viable.—Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture and Texas Agricultural Experiment Station.

*Aerial Microsclerotia of Verticillium Resulting From Conidial Anastomosis.* STEPHEN WILHELM. Certain isolates of the species *Verticillium albo-atrum* Rke. & Bert., form microsclerotia aerially on conidiophores as well as in agar and plant tissue subtrates. The aerial microsclerotia develop by anastomosis of conidia under conditions which permit gradual drying of the watery droplets (heads) into which the conidia are abstricted at the time of their formation. Aerial microsclerotia have been observed on Verticillium isolates growing on several natural straw substrates,

[1] Conners, I. L. 1943. The rusts of saffower. Phytopathology 33: 789-796.

[2] Prasada, R., and H. P. Chothia. 1950. Studies on safflower rust in India. Phytopathology 40: 363-367.

[3] Claassen, C. E., and A. Hoffman. 1950. Safflower production in the western part of the Northern Great Plains. Nebr. Agr. Exp. Sta. Cir. 87.

especially barley, pea, and tomato straw, on potato dextrose agar, and on natural and autoclaved soils. The ability to form aerial microsclerotia appears to be characteristic of only certain individuals of the species.

Prior to becoming microsclerotia, conidia enlarge considerably and send out exceedingly fine tubes by which they become joined. A single conidium may put out as many as 3 or 4 of these delicate tubes (Fig. 1, B). Following anastomosis, the conidial walls become thickened, dark, and carbonized, and the resulting microsclerotium remains upon the conidiophore. (Fig. 1, C, D). Single conidia occasionally enlarge and form resting spores without previous anastomosis with others. These may be considered as chlamydospores, though isolated intercalary chlamydospores are only rarely produced by *V. albo-atrum*.

Conidial anastomosis is well known in *V. albo-atrum*. Reinke and Berthold, authors of the species[1], described and illustrated detached conidia anastomosing shortly after germination. The small microsclerotium and germinating resting cell (their Fig. 1, 3, Table IX) by which they illustrated "Keimende Dauerzellen" are remotely suggestive of aerially formed microsclerotia, though they attributed sclerotial formation to enlargement of chains of thick-walled torulose cells.

The aerial microsclerotia described herein are also somewhat suggestive of the cerebriform type of sclerotium upon which Van Beyma[2] erected the trinomial, *V. dahliae* Klebahn f. *cerebriforme*, though Van Beyma attributed sclerotial production to enlargement and thickening of terminal chains of chlamydospores.

Little is known about the function in nature of conidia of *V. albo-atrum*. Verticillium is a typical member of the Gloiosporae[3] characterized by moist conidia adapted to water and insect dissemination. Its conidia are delicate, succumbing within 3 days in a dry atmosphere at 49–50°C., and within 2 weeks at 40°C. On the other hand, microsclerotia, i.e. those formed in tissue substrates (Fig. 1, A), survive for 6 months at 49–50°C. when dry, and for longer than

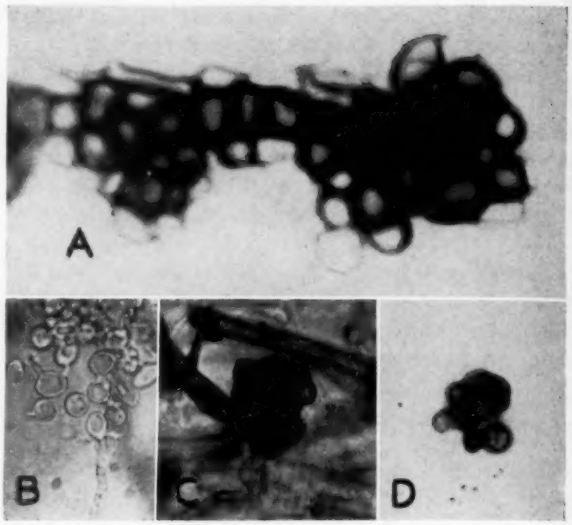


FIG. 1. Comparison of relative size and structure of a typical small microsclerotium formed in plant tissue. A) Aerial microsclerotia formed by conidial anastomosis. B) Swollen anastomosing conidia. Note tubes being produced. C) An anastomosed conidial head becoming thick walled. D) Dislodged aerial microsclerotium. Two conidia are barely visible.

2½ years at 40°C.[4] Microsclerotia in tomato tissue placed on the surface or pushed into moist, nonsterile soil germinate and within a few days become covered with conidial heads. Little or no actual saprophytic growth through the soil takes place[5] but microsclerotial production by anastomosis of the conidia may occur. Such rejuvenation of the fungus may be an important means of survival in soil in the saprophytic phase under environmental conditions which force the germination of microsclerotia. Should aerial microsclerotia be formed on plant debris near the soil surface, they, in addition to the debris itself, may be readily wind-disseminated. Such a mode of dissemination is suggested by the fact that outbreaks of Verticillium wilt have been noted in certain fields of virgin soil brought into cultivation for the first time.[6,7]—Department of Plant Pathology, University of California, Berkeley, California.

[1] Reinke, J., und G. Berthold. 1879. Die Zersetzung der Kartoffel durch Pilze. Untersuchungen aus dem Botanischen Laboratorium der Universität Gottingen. 1: 1-100.

[2] Van Beyma thoe Kingma, F. H. 1939. Ueber einige Formen von Verticillium Dahliae Klebahn. Antonie van Leeuwenhoek. 6: 33-47.

[3] Wakefield, E. M., and G. R. Bisby. 1941. List of Hyphomycetes recorded for Britain. Trans. Brit. Mycol. Soc. 25: 49-126.

[4] Nelson, P. E., and S. Wilhelm. 1953. Thermal death range of *Verticillium albo-atrum*. (Abs.) Phytopathology 43: 589.

[5] Wilhelm, S. 1951. Is *Verticillium albo-atrum* a soil invader or a soil inhabitant? (Abs.) Phytopathology 41: 944-945.

[6] Presley, J. T. 1950. Verticillium wilt of cotton with particular emphasis on variation of the causal organism. Phytopathology 40: 497-511.

[7] Wilhelm, S. 1950. Vertical distribution of *Verticillium albo-atrum* in soils. Phytopathology 40: 368-376.